DEDICATION

This thesis is dedicated to my mother,
Gladys Green, for the sacrifices she made
to provide for my education.

GENETIC MODIFICATION OF SEED FATTY ACID COMPOSITION IN *LINUM USITATISSIMUM* L.

by

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A thesis submitted to the
Australian National University
in fulfilment of the requirements
for the degree of
Doctor of Philosophy

School of Botany
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Canberra, A.C.T.
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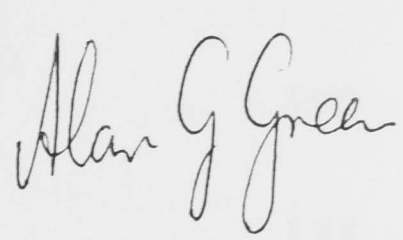
February, 1985

CERTIFICATE OF ORIGINALITY

The text of this thesis contains no material which has been accepted as part of the requirements for any other degree or diploma in any University or any material previously published or written unless due reference to this material is made. Collaboration with others in the collection of data is referred to in the Acknowledgements.



Alan G. Green

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ACKNOWLEDGEMENTS

This study was performed while I was employed as an officer of the CSIRO Division of Plant Industry in Canberra, and the thesis was undertaken through the School of Botany at the Australian National University. I would like to express my gratitude to Dr W. J. Peacock, Chief of the CSIRO Division of Plant Industry, Professor J. Warren-Wilson, Head of the ANU School of Botany, and the Rural Credits Development Fund, for providing the opportunity for me to enrol as a PhD candidate during the course of my CSIRO employment. My deepest appreciation is extended to my University supervisor, Dr P. M. Gresshoff, and my CSIRO supervisors, Dr D. R. Marshall (prior to September 1982) and Dr B. A. Barlow (since September 1982), for their encouragement, guidance and critical evaluation of my research.

I was solely responsible for the design, execution, analysis and reporting of all the research reported in this thesis. Technical assistance was utilised for the screening of the large mutagen-treated population. For this I am indebted to Mr R. Nurzinsky, Mr G. O'Neill and Mr G. Smith, for their very capable work, and to the Oilseeds Research Committee for the provision of funds to support their employment.

The linseed and flax germplasm analysed in this study was provided by Mr G. C. Buzza of the Victorian Department of Agriculture. Wild *Linum* species were obtained from a number of overseas botanic gardens.

I am grateful to the many staff of the CSIRO Division of Plant Industry who have been associated with my research, either directly or indirectly. My thanks extends especially to those members of the Genetic Resources Section who assisted in the planting of the mutagen-treated seed and in the care of plant material.

Chemical analyses were performed in the laboratory of Dr M. L. Tonnet, and her advice on analytical methods, especially gas chromatography, has been greatly appreciated. Statistical advice and some analyses were provided by Mr W. Muller and Dr J. E. Dawson, of CSIRO Division of Mathematics and Statistics. Special thanks are due to Ms A. Warrener, Ms J. Riches and Ms T. Clayton for their skilful preparation of illustrations.

I am most grateful to my wife Rosemary for her patience during the course of this study and for her assistance with the preparation of the manuscript. My father-in-law, Mr A. W. Charles, read the manuscript and provided numerous helpful suggestions on presentation.

SUMMARY

The market for edible vegetable oils is large and continually expanding, but linseed oil cannot currently be utilised in edible products because of the flavour reversion problems associated with its high content of linolenic acid (40-50%). To be suitable for use as an edible oil, linolenic acid would need to be reduced to below 3%. This study was undertaken to determine the extent to which fatty acid composition in *L. usitatissimum* seed oil could be genetically manipulated through the use of natural, interspecific and induced variation, and to evaluate under what environmental conditions edible-quality oil could be produced given appropriate genetic material.

Natural variation for fatty acid composition was assessed in a germplasm collection consisting of 214 flax and linseed accessions. Oleic and linolenic acids varied between 13.3% and 25.2%, and 45.5% and 64.2% respectively, and were strongly negatively correlated within and between all varieties examined. Only limited variation was present for the other fatty acids, palmitic, stearic and linoleic. Parent-offspring correlation analysis revealed significant genetic variation for fatty acid composition within 10 of the 19 accessions studied, indicating a high degree of genetic heterogeneity in the germplasm collection. However, the level of variation was considered to be insufficient to significantly reduce linolenic acid content by intraspecific hybridisation and selection.

In contrast, wild *Linum* species varied widely in the

content of linoleic and linolenic acids in their seed oils. Species closely related to *L. usitatissimum* all had low linoleic and high linolenic acid contents, whereas most species from other sections of the genus were high in linoleic acid (up to 81%) and very low in linolenic (3%). Although these latter species appear to be a valuable genetic resource for breeding low-linolenic genotypes of *L. usitatissimum*, they cannot currently be exploited because of strong reproductive barriers preventing interspecific hybridisation.

Induced mutation techniques were successful in achieving major alterations in the relative proportions of linoleic and linolenic acids in the *L. usitatissimum* cv. Glenelg. Two EMS-derived lines, M1589 and M1722, each contained a single gene mutation that reduced linolenic acid content from 34% to 22% and raised linoleic acid content from 15% to 27%. Genetic analysis revealed that these mutations were in different, unlinked genes and exhibited additive (codominant) gene action. The symbols *Ln1* and *Ln2* are proposed for the mutated genes in M1589 and M1722 respectively.

Recombinant genotypes homozygous for mutant alleles at both loci were very low in linolenic acid (2%) and high in linoleic acid (48%), that is, they had a very low *linoleic desaturation ratio* (LDR). Proportions of the other fatty acids, palmitic, stearic, and oleic acids, and hence *oleic desaturation ratio* (ODR) were unchanged in the mutant lines. The complete inverse correlation between linoleic and linolenic acids in these genotypes indicates that the mutations block linolenic acid synthesis at the linoleic desaturation step.

It is postulated that the *Ln1* and *Ln2* genes represent duplication of a single gene present in the ancestral diploid *Linum* genome, and that loss of function at one or both loci during evolution has resulted in the discrete patterns of variation in linoleic acid and linolenic acid contents observed among the wild *Linum* species. Relatively minor variation in the contents of these fatty acids in high-linolenic linseed varieties is considered to be due to the effects of several minor genes that modify the two major genes.

The comparison of self and reciprocally cross-pollinated seed in crosses between linseed genotypes differing widely in their fatty acid composition revealed that the maternal genotype on which seeds were borne influenced the embryo fatty acid composition. In all crosses oleic acid content was determined by the maternal parent, whereas maternal effects for linoleic and linolenic acids were smaller or absent, depending on the cross. Examination of ODR and LDR demonstrated that oleic desaturation was consistently under maternal control whereas linoleic desaturation was under embryo control. Consequently, selection based on LDR in single seeds can be practised effectively in backcross programs aimed at introducing the *Ln1* and *Ln2* mutants into other adapted linseed genotypes.

A Phytotron study was conducted to determine the effect of temperature during seed maturation on the fatty acid composition of Glenelg and the homozygous mutant genotypes, M1589, M1722 and 'Zero'. In all four genotypes, low temperatures resulted in decreased contents of palmitic, stearic and oleic acids, and increased contents of linoleic and linolenic acids. This was

demonstrated to be due to the greater level of oleic acid desaturation at low temperatures, the subsequent linoleic desaturation step being highly insensitive to temperature. In the 'Zero' genotype, linolenic acid content was below 3% at all temperatures, and linoleic acid content was above 62% when day/night temperature was below 21/16^{o}C. Thus, this genotype should produce a premium quality edible oil similar to sunflower and maize oils when grown as a winter crop in temperate environments.

This study revealed three important differences in oleic and linoleic acid desaturation. Firstly, these two steps are controlled by different genes and can be manipulated independently. Secondly, oleic desaturation is determined by the maternal genotype and linoleic desaturation by the embryo genotype. Thirdly, oleic desaturation is sensitive to temperature, whereas linoleic desaturation is not. Each of these observations is consistent with the generally accepted hypothesis that linolenic acid is produced *via* oleic and linoleic acids by the action of separate substrate-specific desaturase enzymes.

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1.1. BACKGROUND

History of L. usitatissimum *cultivation*

Linum usitatissimum L. is an annual, self-fertilising plant grown either for its fibre (flax, fibre flax) or for its seed oil (oil flax, seed flax, linseed)[1]. It is widely adapted to the temperate climates of the world, although a degree of regional specialisation has occurred. Flax cultivars are taller and less branched and are grown in cool-temperate regions throughout the northern hemisphere, especially in the USSR and eastern Europe. Linseed cultivars are shorter, more branched and larger-seeded and are grown over a wider area in warmer regions such as Argentina, Australia, India, USA, Canada and the USSR.

L. usitatissimum is believed to have originated in either the Middle East or Indian regions (Vavilov, 1951; Durrant, 1976) and spread throughout Asia and Europe, prior to its more recent introduction into the New World. The utilisation of both the fibre and oil can be traced back to early civilisation in Egypt and the Middle East, where linen (the fabric made from flax fibre) was worn as clothing and used to wrap mummies, and the seed oil was used in embalming (Durrant, 1976). Flax fibre remained the principal vegetable fibre in western nations throughout history until the beginning of the present century when cotton took its place. Fibre flax is currently cultivated on approximately 1.3 million hectares worldwide (Sultana, 1983). In the late nineteenth and early twentieth centuries the decline

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1. In this dissertation, 'flax' means varieties grown for their fibre and 'linseed' means varieties grown for their seed oil.

in flax cultivation was paralleled by increased cultivation of linseed as an industrial oilseed crop. In the period 1956-65 average annual world cultivation of linseed was 7.7 million hectares[2].

Utilisation of linseed oil

The industrial quality of linseed oil results from its high content of α-linolenic acid (Table 1). This fatty acid is readily oxidised on exposure to air and imparts a drying quality to the oil which has made it important in the production of paints, varnishes, inks and linoleum. However the market for linseed oil has declined dramatically in recent years as synthetic compounds from the petrochemical industry have increasingly replaced linseed oil in the manufacture of these products. Consequently worldwide production of linseed has steadily declined from 3.6 million tonnes in 1958 to 2.3 million tonnes in 1981.

In contrast to the relatively small and declining demand for industrial vegetable oils, the market for edible vegetable oils is large and continually expanding. In the period 1968 - 1981, world production of edible vegetable oils almost doubled from 23 to 44 million tonnes (Boelhouwer, 1983). The discovery that polyunsaturated fatty acids are essential components in human nutrition, and are of presumptive value in the prevention of heart and vascular diseases, has promoted the production and application of vegetable oils such as safflower, sunflower and

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2. Production and cultivation figures compiled from various editions of the F.A.O. Production Yearbook

soybean, with high contents of linoleic acid (Table 1). However, linolenic acid, although also a polyunsaturated fatty acid and an essential dietary component for humans, is considered an undesirable component of edible oils because of its chemical instability. Hence linseed oil is not currently used in edible products.

Table 1: Fatty acid composition of linseed and major edible oilseeds (A.O.F., 1982).

	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)
Safflower	7	3	12	78	--
Sunflower	6	2	26	66	--
Cottonseed	22	2	20	56	--
Peanut	10	3	60	22	--
Maize	12	2	26	59	1
Soybean	10	3	24	55	8
Rapeseed (Canola)	4	1	60	25	10
Linseed	6	4	18	19	53

Undesirability of linolenic acid in edible oils

The major problem with edible products made from oils containing linolenic acid is their tendency to produce off-flavours or to become rancid during storage. The poor flavour stability of soybean oil has long been attributed to the presence of linolenic acid (Durkee, 1936; Dutton *et al.*, 1951). There is usually 7-8% of this acid in soybean oil, compared with less than 1% in more stable oils, such as maize and cottonseed oil. The role of linolenic acid in flavour reversion was shown

experimentally by Sanders (1946) who added 10 parts of linolenic acid obtained from linseed oil to 90 parts of cottonseed fatty acids, esterified the mixture to a finished oil, and observed that its flavour instability characteristics were very similar to those of soybean oil. Experiments in which the linolenic acid content of soybean oil has been varied suggest that the flavour stability of the oil increases as the linolenic level decreases below 3%, but for good flavour stability a linolenic acid content below 1% is necessary (Okkerse *et al.*, 1967; Cowan *et al.*, 1970).

In oils containing relatively low levels of linolenic acid, such as soybean and rapeseed (Table 1), flavour reversion can be prevented by the process of hydrogenation in which linolenic acid is converted to more stable, less unsaturated fatty acids. However this procedure is not of practical value for reducing the content of linolenic acid in linseed oil since it is costly, non-specific, and can lead to the production of nutritionally inferior *trans* isomers (Wilcox *et al.*, 1984), as well as another flavour reversion factor, iso-linoleic acid (Smouse, 1979). A more satisfactory and permanent way to convert linseed oil to an edible oil would be the genetic removal of linolenic acid from linseed, or at least to reduce it to below the 1% level believed to be sufficient to avoid flavour reversion.

Biosynthesis of linolenic acid

An understanding of the biosynthetic pathway leading to the synthesis of linolenic acid in seed tissue is essential for a consideration of the potential for its genetic removal.

Dutton and Mounts (1966) exposed flax, soybean and safflower plants to $^{14}CO_2$ at the seed-set stage and measured the radioactivity of the seed triglycerides at various intervals of time. Of the C18 unsaturated acids, oleic was the first to acquire radioactivity, which subsequently appeared in linoleic acid and then in linolenic acid. On the basis of the radioactivity-time curves, they concluded that the polyunsaturated fatty acids, linoleic and linolenic, are formed by the consecutive desaturation of oleic acid in all three species. In a similar study, Cherif *et al.*, (1975) incubated plant tissue with radioactively-labelled oleate and linoleate and observed the incorporation of radioactivity into linolenate. Likewise, they concluded that the desaturation pathway is the major route for the biosynthesis of α-linolenic acid in leaves, roots and seeds of several higher plants, including flax.

Alternative pathways for linolenic acid synthesis, such as by direct desaturation of oleic acid (Kannangara and Stumpf, 1972) or by elongation of dodecatrienoic acid (Kannangara *et al.*, 1973), have been demonstrated to operate in photosynthetic tissue but are not considered to account for the large amount of linolenic acid present in linseed oil (Oulaghan and Wills, 1976).

Although a small proportion of the fatty acids present in seed oils are essential for specific cellular functions, such as in the structure of cell membranes or as enzyme cofactors, the great majority are present as triglycerides, condensed into oil-bodies known as oleosomes (Appelqvist, 1980). The function

of these triglycerides is to provide a source of carbon skeletons and energy during germination, requirements which are largely independent of their specific fatty acid composition. It should thus be possible to alter the fatty acid composition of seed triglycerides, providing that such changes do not affect other cellular lipids *via* common pools of precursors or biosynthetic pathways (Robbelen, 1982).

Genetic modification of fatty acid composition in oilseeds

The relatively minor chemical changes required to interconvert individual fatty acids, combined with the apparent lack of functional constraints on triglyceride fatty acid composition, have enabled this character to be readily manipulated by a variety of plant breeding techniques. Selection within naturally-occurring variation in rapeseed (*Brassica napus*) resulted in the elimination of the nutritionally-undesirable erucic acid and its precursor eicosenoic acid, and converted this crop into a major source of edible oil (Stefansson *et al.*, 1961; Downey and Harvey, 1963). Zero-erucic genotypes have since been developed in the related species *B. campestris* (Downey, 1964a; Jonsson, 1973), and *B. juncea* (Kirk and Oram, 1981) using the same methodology. Similarly, selection among existing within-species variation has resulted in the production of high-oleic genotypes of safflower (Knowles, 1968) and sunflower (Downey and Dorrell, 1971) both of which are normally high-linoleic types. By contrast, natural within-species variation has not been large enough to enable the elimination of linolenic acid from either soybean or rapeseed, but breeders have made progress in reducing linolenic acid

content in both species through mutation breeding (Hammond *et al.*, 1972; Rakow, 1973; Robbelen and Nitsch, 1975; Wilcox *et al.*, 1984). More recently, Roy and Tarr (1984) reported the use of the related species *B. juncea* as a source of genes to reduce linolenic acid content in rapeseed (*B. napus*).

Breeding for low linolenic acid content in linseed

Each of these sources of variation, namely, natural, interspecific and induced, has in the past been suggested as a possible means of eliminating linolenic acid from linseed oil. Dorrell (1972) reported the occurrence of natural variants having as little as 13% linolenic acid, although these lines proved to be unstable. It has often been suggested that the many wild *Linum* species that have very low levels of linolenic acid in their seed oils (Plessers, 1966; Yermanos, 1966; Yermanos *et al.*, 1966) offer a potentially valuable genetic resource for the improvement of the cultivated species, but there appears to have been no concerted effort to assess the extent to which this resource may be exploited through interspecific hybridisation. Mutation breeding experiments in linseed have demonstrated increased variation in fatty acid composition (Rath and Scharf, 1968; Vereshchagin, 1973; Marquard *et al.*, 1978) but do not appear to have been directed to the selection of low-linolenic acid genotypes. In view of these reports and of the outstanding success in genetically modifying this character in other oilseed species, it seems reasonable to postulate that fatty acid composition in linseed could be genetically manipulated, given adequate experimentation.

1.2. AIMS OF RESEARCH

The aim of this research project was to assess the extent to which linolenic acid content of linseed oil could be genetically modified through the use of natural, interspecific and induced variablility; and to evaluate under what environmental conditions edible quality oil could be produced, given appropriate genetic material. The experimental approach was designed to provide detailed information on the following:-

1. The level, organisation and genetic basis of natural variation in seed fatty acid composition within *L. usitatissimum*.

2. The level of natural variation in seed fatty acid composition between wild *Linum* species; and the prospects for incorporation of their desirable characteristics into *L. usitatissimum*.

3. The interrelationships between individual fatty acids.

4. The degree of mutability of fatty acid composition in *L. usitatissimum*.

5. The inheritance pattern of any induced mutants.

6. The response of fatty acid composition in normal and mutant lines to the principal environmental determinant of quality, i.e. temperature during seed maturation.

7. The detection of maternal effects on seed fatty acid composition and their importance in breeding methodology.

The experiments conducted are presented in Chapters 2 to 5. Each chapter contains a review of relevant literature (Introduction) and a Materials and Methods section outlining experimental details specific to that chapter. Methods common to all chapters are given in the next section of this chapter (1.3.). Chapter 6 provides general discussion and conclusions. Fatty acid composition data for the germplasm collection is presented in an appendix.

During the course of the study papers were submitted for publication in journals. The assessment of natural variability (Chapter 2) was published as two papers (Green and Marshall, 1981; Green, 1983) and the isolation of linolenic acid mutants (Chapter 3) was also reported (Green and Marshall, 1984). Chapters 3, 4 and 5 are expected to form the basis of three further manuscripts.

1.3. METHODS

Unless otherwise specified, plants were grown singly in pots containing a 3:1 mixture of potting mix and coarse sand. Experiments were conducted in a glasshouse under natural photoperiod but with temperature controlled at 27^{o}C (12 hr day)/ 20^{o}C (12 hr night). Analyses of seed characters throughout the study were performed by the routine procedures outlined below. The amounts of seed used in analyses varied between experiments, depending on availability, and are indicated in the relevant chapters.

Seed weight (abbreviated to 'seedwt' in some tables) is

expressed as grams per hundred seeds and was determined by weighing a sample of 30 seeds following oven-drying (100^{o}C) for 16 hours. Oil content was measured non-destructively on this sample by wide-band nuclear magnetic resonance (NMR) in a Quantity Analyser (Newport Instruments) with reference to a standard of pure linseed oil.

Fatty acid composition was determined on either bulk seed samples, individual whole seeds or half-seeds (single cotyledons). Analyses reported in Chapter 2 were conducted on intact seed since initial experiments indicated that at least 65% of the total oil content of the intact seed was recovered as fatty acid methyl esters by this method. Further, Yermanos (1972) demonstrated that when flax seeds were soaked in organic solvents the fatty acids that leached out were a representative sample of the total seed fatty acid composition. However, later experiments with mutant lines in the current study revealed significant differences in embryo and endosperm composition and a bias for intact seeds analyses towards the composition of the endosperm (outer) fraction. Thus all subsequent bulk seed analyses were performed on samples that had been crushed between filter paper discs to extrude the oil and allow complete solvent penetration. This method provided reliable unbiased results.

Half-seed analyses were performed where it was necessary to establish progeny plants from analysed seeds. Seeds were germinated on moist filter paper in petri-dishes kept in the dark at room temperature (approx. 25^{o}C). Previous experiments by Huber and Zalik (1963) demonstrating that fatty acid composition of flax seed did not change significantly during the first four

days of germination were confirmed using the cultivar Glenelg. At four days one cotyledon was excised for analysis and the remaining seedling transplanted to soil. In Chapter 5, where progeny tests of single seeds were not required, the whole embryo (testa and endosperm removed) was analysed as a single sample.

For all samples, fatty acid methyl esters were prepared using a modification of the method of Welch (1977). Bulk seed samples were transmethylated in 30 ml Macartney bottles containing 5 ml of 2% methanolic sulphuric acid, for 3 hours at 80^{o}C. For single seed and half-seed samples, reagent volumes were halved and smaller (9 ml) Macartney bottles used as reaction vessels. After cooling, methyl esters were extracted into 5 ml of petroleum ether, washed twice with 2% potassium bicarbonate to neutralise any remaining acid, and dried over sodium sulphate. The ether extract was then transferred to a micro-vial for analysis by gas chromatography. Where samples were stored prior to analysis they were first evaporated to dryness under nitrogen and then kept at 2^{o}C.

Fatty acid methyl esters were analysed by gas chromatography using either of two systems. Analyses reported in Chapter 2.2 were performed on a Varian Aerograph 204B chromatograph. All subsequent analyses were performed on a Varian Model 3700 gas chromatograph. Both chromatographs were equipped with flame ionisation detectors. Stainless steel columns (length 360 cm, internal diameter 2 mm) were used throughout and were packed with either Silar 10C (10%) or EGSS-X (15%) on Gas Chrom Q. The oven, detector and injector

temperatures were respectively 185, 200 and 230°C (Chapter 2.2.) 210, 280 and 280°C (Chapter 2.3.) and 180, 260 and 260°C in all other experiments. Nitrogen was used as the carrier gas. Percentages of fatty acid methyl esters were calculated by a Varian CDS 111C Data System with reference to standard mixtures obtained from Applied Science Laboratories Inc., and measured on the same column under identical conditions. Fatty acid data are presented throughout as percentage of total fatty acids. For brevity in table headings the standard nomenclature for fatty acids given in Table 2 has been used. Where mentioned by name in text and tables, 'fatty acid residues' are invariably referred to as 'fatty acids', that is, oleate is referred to as oleic acid, linoleate as linoleic acid, etc.

An alternative method of assessing the composition of unsaturated fatty acids is through the ratios ODR (*oleic desaturation ratio*) and LDR (*linoleic desaturation ratio*), derived by the following formulae:-

$$\text{ODR} = \frac{\text{linoleic acid (\%)} + \text{linolenic acid (\%)}}{\text{oleic acid (\%)}}$$

$$\text{LDR} = \frac{\text{linolenic acid (\%)}}{\text{linoleic acid (\%)}}$$

The magnitudes of these ratios are directly proportional to the activities of the individual enzyme systems believed to be responsible for the desaturation of oleic acid and linoleic acid respectively.

Statistical analyses performed throughout the thesis have used standard techniques referred to in Steele and Torrie (1960).

Table 2: Nomenclature for the principal fatty acids present in *Linum* seed oils.

Nomenclature [a]	Common name	Systematic name
Saturated		
C16:0	Palmitic	Hexadecanoic
C18:0	Stearic	Octadecanoic
Mono-unsaturated		
C18:1	Oleic	*cis*-9-octadecenoic
C18:1-OH	Ricinoleic	*cis*-12-hydroxy-9-octadecenoic
Poly-unsaturated		
C18:2	Linoleic	*cis*-9,12-octadecadienoic
C18:3	Linolenic	*cis*-9,12,15-octadecatrienoic

[a] number of carbon atoms : number of double bonds

CHAPTER TWO

NATURAL VARIATION FOR SEED FATTY ACID COMPOSITION IN THE GENUS *LINUM*

2.1. INTRODUCTION

Extensive natural variation in fatty acid composition has been observed in almost every economic oilseed species. Moreover, it has become evident that although environment can be a significant factor in causing variation, the basic differences are due to genetic control of the biochemical processes of oil synthesis (Downey and Dorrell, 1971). Because of this, fatty acid composition has been successfully modified in several oilseed crop species through the selection of naturally-occuring variants. Both safflower and sunflower, traditionally low-oleic, high-linoleic oilseeds, have been modified to produce high-oleic, low-linoleic oils (Knowles, 1968; Downey and Dorrell, 1971). Zero-erucic acid rapeseed and mustard genotypes have been developed by selection within high-erucic (40-60%) varieties of *Brassica napus* (Stefansson *et al.*, 1961), *B. campestris* (Downey, 1964a), and *B. juncea* (Kirk and Oram, 1981).

Where the level of variation between and within lines has been insufficient to achieve selection goals, it has sometimes been possible to incorporate suitable genes from related species. Recently this approach was used to select rapeseed genotypes having reduced levels of linolenic acid from within *B. napus* x *B. juncea* hybrids (Roy and Tarr, 1984).

To assess whether existing genetic variation is sufficient to enable the development of low-linolenic acid linseed genotypes it is first necessary to determine the extent and genetic basis of natural variation in seed oil fatty acid composition, both between and within *L. usitatissimum* lines and

in related species that may be a potential genetic resource.

Zimmerman and Klosterman (1959) analysed the world collection of 1175 flax and linseed lines for seed fatty acid composition. Linolenic acid was the major constituent, varying from 45% to 65% of the oil. Oleic acid ranged from 10% to 28% and was strongly negatively correlated with linolenic acid content. Linoleic acid ranged from 7% to 19%, with palmitic and stearic acids accounting for the remainder. A proportion of this variation was considered to be due to environment, since the material analysed originated from a number of locations from different latitudes, and control varieties grown at the same locations varied significantly in their fatty acid composition.

The full extent of genetic variation in this collection was not evident purely from an examination of variety means, since extensive within-line variation was present. Fifteen individual plants from each of five linseed lines were analysed and wide variability was found in oleic acid and linolenic acid contents, the latter ranging from 38.2% to 55.7% within one variety. Although the genetic basis for this variation was not determined, it was concluded that significant heterogeneity existed for fatty acid composition within the World Collection.

Dorrell (1972) reported even greater within-line variation for fatty acid composition. By analysing single seeds of the linseed cultivar Ward and selecting for extreme fatty acid composition, lines were produced ranging from 14% to 79% in oleic acid content and from 13% to 69% in linolenic. However, the genetic basis of this variation was not established and these lines displayed considerably reduced variation in later

generations (Dorrell, 1972).

Wild *Linum* species have often been suggested as a means of reducing linolenic acid levels in the cultivated species through interspecific hybridisation (Yermanos, 1966; Dorrell, 1972). Plessers (1966) and Yermanos *et al.* (1966) reported extensive variation in fatty acid composition in a wide range of wild *Linum* species. Two main types were evident; one with high linolenic and low linoleic, similar to *L. usitatissimum*, and a contrasting type having low levels of linolenic acid and very high levels of linoleic acid, a combination not found in *L. usitatissimum*. Some species in the latter group had as little as 1% linolenic acid and appear to be obvious genetic resources for improvement of the cultivated species.

Kleiman and Spencer (1971) found that ricinoleic acid, a hydroxylated derivative of oleic acid, accounted for 15% of the seed oil of an accession of *L. mucronatum* collected in Turkey. Although ricinoleic acid is the principal component of the oil from castor bean (*Ricinus communis*), it is only rarely a component of other seed oils and, when present, is usually in much lower proportion than the more common saturated, mono-unsaturated and C18 polyunsaturated fatty acids. The previous extensive surveys of fatty acid composition in *Linum* (Yermanos, 1966; Plessers, 1966), although including samples of *L. mucronatum* and several closely related species, had not identified ricinoleic acid as a component of the seed oil. The occurrence of only one accession from a single species possessing the biosynthetic pathway necessary for the production of ricinoleic acid seems unlikely. An alternative explanation

could be that the earlier surveys did not adequately test for this unusual fatty acid. Therefore suitable chromatographic techniques were employed in the current study to detect the presence of ricinoleic acid in all species investigated.

The research reported in this chapter was directed towards further evaluation of the extent and genetic basis of natural variation in fatty acid composition between and within *L. usitatissimum* lines, and in related wild *Linum* species, with a view to assessing the prospects of reducing linolenic acid content in linseed by selection within *L. usitatissimum* germplasm or appropriate interspecific hybrids. The extent of genetic variation for fatty acid composition was examined in a collection of linseed and flax cultivars, introductions and breeding lines grown in a common environment. A number of cultivars, including Ward, were examined in greater detail using the parent-offspring correlation method to determine whether within-variety differences were genetic in origin. The likely correlated responses to selection for reduced linolenic acid content were also considered.

2.2. MATERIALS AND METHODS

Seeds of 31 *Linum* species representing all five sections of the genus were obtained from various sources. The taxonomic distribution of these accessions, following the treatment of Rogers (1972), is presented in Table 3. A total of 169 accessions were analysed, including a sample of the *L. mucronatum* genotype (CPI 82684) found to contain ricinoleic acid

by Kleiman and Spencer (1971). Analyses for fatty acid composition were performed directly on samples of the introduced seed, ranging from 20 to 200 seeds depending on size and availability. The oil in the samples was transmethylated without prior extraction, using the methods outlined in Chapter 1.3. Because of the very small seed weight and reported low oil percentage of most of the wild species (Yermanos *et al.*, 1966) no attempt was made to measure these characters.

To examine variation within *L. usitatissimum*, a germplasm collection consisting of 201 linseed and 13 flax cultivars and breeding lines of diverse origin was analysed. This material, provided by the Victorian Department of Agriculture, had been grown in the spring of 1976 in unreplicated 5 m rows at Noorat in western Victoria, all plants within the rows being harvested and the seed bulked. A sample of 30 seeds was taken from each accession for analysis in this study.

Repeatability of within-line variation for fatty acid composition was examined by parent-offspring correlation. For each of 19 linseed varieties listed in Table 6 (page 37), 30 plants were planted in 25 cm diameter pots in a glasshouse in Canberra in May 1978. Plants were allowed to self-pollinate and were harvested separately at maturity. From each plant, a sample of 30 seeds was taken for analysis and a single seed planted in the same glasshouse under similar conditions. Again, selfed seeds were harvested from these plants and 30 seeds taken for analysis. Thus, for each of the 19 varieties, seed from 30 parent-offspring pairs of plants were available for chemical analysis. Seed weight, oil content and percentage fatty acid

composition were determined on the 30-seed samples using the methods outlined in Chapter 1.3.

Seed of the cultivar Ward, reported to be highly variable for fatty acid composition, was obtained from Dr G. Dorrell (Agriculture Canada) and four single plants were grown under quarantine glasshouse conditions. Six whole seeds from each plant were analysed separately for fatty acid composition. The most variable plant was identified and a further 62 seeds analysed using the half-seed technique described in Chapter 1.3. Progeny plants established from these seeds were harvested at maturity and 30-seed samples from each plant analysed for fatty acid composition.

2.3. RESULTS

Variation between Linum *species*

The mean fatty acid compositions of *Linum* species seed oils are presented in Table 3. Palmitic and stearic acids were minor components in all species, averaging 8% and 3% of total fatty acids respectively, with no significant differences between sections. However, taxonomic relationships were evident for the principal fatty acids, namely oleic, linoleic and linolenic. In species from the sections *Linastrum*, *Cathartolinum* and *Syllinum*, linoleic acid was the major component, ranging from 46% to 82%. Oleic acid varied between 8% and 24%, and linolenic between 3% and 28% in these species. By contrast, the major component of species from the sections *Linum* and *Dasylinum* was linolenic acid, ranging from 38% to 57%, with

Table 3: Fatty acid composition of *Linum* seed oils.

Species[a]	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	18:1OH	Unknown[b]
Section *Linum*							
L. usitatissimum (7)	9.3	2.1	17.2	19.1	52.2	-	+
L. angustifolium (4)	11.1	3.5	17.9	14.5	53.0	-	+
L. bienne (11)	11.6	4.4	16.9	14.7	52.5	-	+
L. grandiflorum var. *rubrum* (10)	9.7	3.8	21.5	18.6	46.4	-	++
L. marginale (41)	6.5	2.0	15.5	19.0	57.1	-	-
L. perenne (6)	7.5	2.2	22.5	28.1	39.8	-	++
L. alpinum (6)	7.7	2.3	20.5	27.9	41.7	-	++
L. extraaxillare (1)	7.3	2.0	12.1	28.0	50.7	-	+
L. anglicum (3)	7.0	2.4	14.3	26.0	50.5	-	+
L. austriacum (13)	7.7	3.0	21.5	28.6	39.2	-	++
L. leonii (2)	6.4	1.9	24.3	43.7	23.8	-	+
L. lewisii (1)	7.7	2.3	20.1	25.4	44.4	-	+
L. altaicum (1)	8.6	2.4	22.6	24.3	42.2	-	+
L. mexicanum (1)	8.7	2.3	20.7	28.1	40.3	-	+
L. narbonense (1)	6.6	1.8	22.0	32.2	37.5	-	+
Section *Dasylinum*							
L. hirsutum (2)	6.6	1.8	19.4	27.4	45.0	-	+
L. viscosum (2)	7.2	1.1	13.4	28.2	50.2	-	+
Section *Cathartolinum*							
L. catharticum (5)	7.6	2.8	13.2	64.3	12.1	-	-
Section *Linastrum*							
L. maritimum (3)	11.0	3.0	13.8	46.1	26.1	-	-
L. strictum (3)	8.9	3.1	7.6	52.9	27.6	-	-
L. rigidum (1)	7.7	1.3	8.1	62.1	20.8	-	-
L. sulcatum (1)	7.9	2.5	12.5	68.7	8.5	-	-
L. imbricatum (1)	8.9	2.5	6.6	75.4	6.5	-	-
L. lundelli (1)	8.4	2.4	8.9	74.6	5.7	-	-
L. tenuifolium (6)	5.0	2.1	8.0	81.5	3.6	-	-
L. salsoloides (3)	5.9	2.7	9.6	78.5	3.4	-	-
Section *Syllinum*							
L. flavum (8)	7.7	3.7	23.8	47.6	12.6	4.5	-
L. arboreum (2)	6.5	2.9	23.1	50.9	13.6	3.1	-
L. dolomiticum (1)	5.9	2.8	17.8	53.3	16.5	3.6	-
L. campanulatum (2)	5.3	2.3	21.8	51.2	16.4	3.2	-
L. mucronatum (2)	7.2	3.0	20.9	60.8	3.2	5.1	-

[a] Number in brackets indicates number of accessions analysed.
[b] Unidentified fatty acid with ECL of 27.2 (see text)
- = absent, + = less than 5%, ++ = greater than 5%

approximately equal proportions of oleic (12%-23%) and linoleic acids (15%-32%). *L. leonii* (Section *Linum*) was the single exception to this pattern, having a high concentration of linoleic acid (44%) with equal amounts of oleic and linolenic acids (24%). Across the genus as a whole, linoleic and linolenic acid contents were strongly negatively correlated (r = -0.95). The results for these fatty acids are in general agreement with those of previous studies (Plessers, 1966; Yermanos, 1966).

An unknown fatty acid was detected in all species from sections *Linum* and *Dasylinum*, with the exception of *L. marginale*. The methyl ester of this fatty acid was eluted a considerable time after linolenic acid and had an equivalent chain length (ECL) of 27.2 under the chromatographic conditions employed in this study. It could not be identified by reference to available fatty acid methyl esters, including those of C20:0, C20:1, C22:0, C22:1, C24:0 and C24:1 and hence could not be precisely quantified. However it accounted for between 1% and 8% of the total chromatogram peak area. This compound was not detected in any species from other sections of the genus and has not been previously reported in the literature. Its absence from *L. marginale* supports the recent suggestion of Rogers (1984) that this species be taxonomically separated from most other species in sect. *Linum*.

Ricinoleic acid was present as a minor component of all five species tested in the section *Syllinum*, ranging from 3% to 8% of total fatty acids, whereas no species from the remainder of the genus contained any detectable level. Although one of the *L. mucronatum* accessions analysed (CPI 82684) had been

reported to contain ricinoleic acid (Kleiman and Spencer, 1971), previous analyses of other species from this section had failed to detect it (Plessers, 1966; Yermanos, 1966). This could be due to the fact that under the chromatographic conditions normally used to analyse fatty acid methyl esters of seed oils, ricinoleic acid has a retention time approximately four times that of linolenic acid. Thus, in studies that were not specifically searching for ricinoleic acid, the chromatographic analysis may have been terminated prior to the elution of this compound. Such an explanation could also account for the previous failure to detect the unidentified fatty acid observed in the present study.

Variation within Linum usitatissimum

Fatty acid composition data for all individual entries in the germplasm collection are presented in Appendix 1. Analysis of the 214 linseed and flax lines revealed considerable variation in seed weight, oil content and fatty acid composition (Table 4). Flax lines generally had smaller seeds and lower oil contents than linseed lines, but there were no substantial differences between the two groups in fatty acid composition. The minimum linolenic acid content of linseed lines was 45.5% in a breeding line derived from the cultivar Argentina, this level being considerably below that of the lowest flax line (55.7% linolenic). However, no lines were identified that had substantially less linolenic acid than Glenelg, the current Australian linseed cultivar.

Although the range of expression for each character did not exceed that previously reported (Zimmerman and Klosterman

1959; McGregor and Carson, 1961), lines were found with considerably greater oil content than the current Australian cultivar Glenelg. The brown-seeded cultivar Dunes and the yellow-seeded Avantgarde, with an average oil content of 44%, were notable in this regard and could be useful sources of genes for the improvement of oil content in cultivars adapted to Australian conditions.

Table 4: Mean, minimum and maximum values for seed weight, percentage oil and fatty acid composition, in 201 linseed and 13 flax lines.

	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Linseed									
Minimum	393	34.6	3.8	1.3	13.3	10.4	45.5	2.63	2.46
Mean	636	41.9	7.0	3.3	18.6	16.3	55.0	3.90	3.43
Maximum	1009	46.4	9.2	6.2	25.2	20.9	63.1	5.80	5.88
Flax									
Minimum	360	33.3	4.6	1.5	14.3	13.6	55.7	3.66	3.11
Mean	459	37.4	5.1	2.7	17.2	15.8	59.3	4.43	3.80
Maximum	536	42.2	5.6	3.8	20.0	17.9	64.2	5.48	4.55

Correlations between characters are presented separately for linseed and flax groups in Table 5. Among linseed lines, the larger-seeded types had higher oil, palmitic, stearic and oleic acid contents, but lower linolenic and ODR, than the smaller-seeded types. Oil content was positively correlated with palmitic, stearic and oleic acid content, and negatively correlated with linoleic and linolenic acids and ODR. Linolenic was negatively correlated with all other fatty acids and

positively correlated with both ODR and LDR, these latter two characters also being positively correlated. Fewer significant correlations were present among flax lines, where higher oil content tended to be associated with higher stearic acid, lower linoleic acid and higher LDR. Among individual fatty acids, the correlation of greatest magnitude in both flax and linseed groups was that between oleic acid and linolenic acid (r = -0.77 in linseed lines; r = -0.93 in flax lines).

Table 5: Correlations between seed weight, percentage oil and fatty acid composition in linseed varieties (above diagonal) and flax varieties (below diagonal).

	Seed weight	Oil	Fatty acid 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Seed weight		0.46*	0.34*	0.28*	0.30*	-0.08	-0.32*	-0.35*	-0.07
Oil	0.35		0.23*	0.23*	0.26*	-0.21*	-0.18*	-0.30*	0.09
16:0	0.51	0.07		0.53*	0.12	-0.13	-0.44*	-0.24*	-0.07
18:0	0.34	0.65*	0.22		0.28*	-0.20*	-0.53*	-0.41*	-0.08
18:1	-0.03	-0.39	-0.22	-0.46		0.07	-0.77*	-0.97*	-0.35*
18:2	-0.40	-0.71*	-0.24	-0.74*	0.54		-0.46*	-0.07	-0.92*
18:3	0.10	0.52	0.14	0.47	-0.93*	-0.76*		0.83*	0.73*
ODR	-0.08	0.35	0.15	0.34	-0.99*	-0.48	0.92*		0.37*
LDR	0.31	0.68*	0.21	0.67*	-0.68*	-0.98*	0.87*	0.63*	

* Significant at the 5% level.

For each character studied, the within-line parent-offspring correlation coefficient is an indicator of the presence or absence of genetic variation for that character. If differences between the performance of the 30 plants in the parental generation were due solely to random environmental effects, then the performance of the offspring generation, grown

in a similar environment, should not have been correlated significantly with parental performance. However, if some or all of the differences in the parental generation were due to genetic causes, then significantly positive parent-offspring correlations could be expected, the size of the correlation being dependent on the relative magnitudes of genetic and environmental effects. Of the 19 lines studied in this manner (Table 6) significant parent-offspring correlations were found in ten for seed weight, three for oil content, ten for fatty acid composition (assessed as significant correlations for three or more fatty acids), seven for ODR and twelve for LDR. Two varieties, Boladi and Dunes, had significant correlations for all five characters, whereas two varieties, Glenelg and CPI 77206, had no significant correlations for any character.

If genotype x environment interactions are present between the parental and offspring generations, then it is possible that non-significant correlations will be obtained in spite of the presence of significant genetic variation. In order to test for the presence of such interactions, the means of all parental plants and offspring plants were calculated for each line, and the correlation of these means across lines determined. Highly significant correlation coefficients were found for all characters, *viz.* seed weight (r = 0.92; $P < 0.01$); oil content (0.76; 0.01); each of the fatty acids, palmitic (0.46; 0.01), stearic (0.37; 0.05), oleic (0.65; 0.01), linoleic (0.95; 0.01), linolenic (0.82; 0.01); ODR (0.70; 0.01); and LDR (0.97; 0.01). Thus no large differences were evident in the relative performance of lines in the parental and offspring generations, and it is reasonable to assume that this was also true for

Table 6: Significant (5% level) within-line parent-offspring correlation coefficients for seed weight, percentage oil and fatty acid composition.

Variety	Seed weight	Oil	Fatty acid 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Heterogeneous varieties									
Boladi	0.76	0.48	0.44	-	0.41	0.36	0.36	0.44	0.40
Cree	0.66	-	-	0.71	0.45	0.62	0.39	0.49	0.66
Dunes	0.65	0.37	-	-	0.44	0.72	0.40	0.57	0.53
Hazeldean	0.45	-	-	0.41	0.69	0.64	0.75	0.72	0.67
Kameniza	-	-	0.49	-	0.75	0.38	0.68	0.61	0.54
Linfola 2	-	-	-	0.56	-	0.38	0.42	-	0.61
Linfola 3	0.37	-	-	0.67	-	0.62	0.59	-	0.74
Linfola 4	-	0.54	0.40	0.37	-	0.50	0.52	0.42	0.44
New River	0.75	-	0.49	-	-	0.54	0.56	-	0.63
CPI 77209	-	-	-	-	0.54	0.45	0.45	0.47	0.62
Homogeneous varieties									
Avantgarde	0.85	-	-	-	-	-	-	-	-
Calar	0.55	-	0.37	-	-	0.39	-	-	-
Glenelg	-	-	-	-	-	-	-	-	-
Linott	0.73	-	0.69	0.88	-	-	-	-	-
Somaco	-	-	-	-	-	-	-	-	0.67
Walsh	0.61	-	-	0.50	-	0.37	-	-	-
CPI 72377	-	-	-	0.38	-	0.44	-	-	-
CPI 77206	-	-	-	-	-	-	-	-	-
CPI 77207	-	-	-	-	-	-	-	-	0.41

relative performance of genotypes within lines. The parent-offspring correlation method used to detect the presence of genetic variation is therefore not seriously confounded by genotype x environment interactions in this study.

Between-character correlations were calculated within each line in both the parental and offspring generations (Table 7). Oil content was positively correlated with seed weight in either the parental or offspring generation in nine lines, and in both generations in a further three. No significant negative correlations were found between these two characters. Both positive and negative correlations were present between seed weight and each of the fatty acids in both generations, with the direction of the correlation occasionally differing between the two generations within the one line. Two cultivars, Hazeldean and New River, had significant genetic variation for seed weight and linolenic acid (Table 6) and a negative correlation between these two characters in both generations, indicating that selection within these cultivars for reduced linolenic acid would indirectly select lines with above-average seed weight, and *vice versa*.

Few significant correlations were evident between oil content and fatty acid composition. Oil content was associated positively with linoleic acid and negatively with oleic acid in Glenelg, Linfola 2, Linfola 4 and Somaco, but the association between oil content and oleic acid was positive in CPI 77206. There were no significant correlations between oil content and linolenic acid in 16 of the 19 lines, including Boladi and Dunes for which genetic variability existed for both characters. Thus

Table 7: Frequency of correlations between characters within each of the 19 lines in the parental (P) and offspring (O) generations.

Correlation		Seed weight *vs*						Oil *vs*				
P	O	Oil	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
+ve	+ve	3	1	2	3	-	-	-	-	1	1	-
+ve	-ve	-	-	-	-	-	-	-	-	-	-	-
+ve	ns	8	1	2	4	5	3	1	1	-	3	1
-ve	+ve	-	-	-	1	-	1	-	-	-	-	-
-ve	-ve	-	-	-	-	-	2	-	-	1	-	1
-ve	ns	-	1	3	3	1	4	2	-	4	-	-
ns	+ve	1	4	-	-	3	-	-	3	-	1	-
ns	-ve	-	1	-	-	1	1	-	-	1	-	1
ns	ns	7	11	12	8	9	8	16	15	12	14	16
Total	+ve	15	7	6	11	8	4	1	4	2	6	1
Total	-ve	-	2	3	4	2	10	2	-	7	-	3
Total	ns	23	29	29	23	28	24	35	34	29	32	34

Correlation		16:0 *vs*				18:0 *vs*			18:1 *vs*		18:2
P	O	18:0	18:1	18:2	18:3	18:1	18:2	18:3	18:2	18:3	18:3
+ve	+ve	2	1	1	-	2	1	2	-	-	-
+ve	-ve	1	-	-	1	-	-	-	-	-	2
+ve	ns	1	3	-	-	2	-	-	-	-	-
-ve	+ve	-	-	-	-	2	-	-	1	-	-
-ve	-ve	-	-	-	8	-	1	4	3	19	4
-ve	ns	-	2	2	3	-	4	2	6	-	-
ns	+ve	6	4	5	-	4	4	-	2	-	1
ns	-ve	-	-	-	4	-	-	5	2	-	10
ns	ns	9	9	11	3	9	9	6	5	-	2
Total	+ve	11	9	7	1	12	6	2	3	-	3
Total	-ve	1	2	2	24	2	6	17	15	38	20
Total	ns	26	27	29	13	24	26	19	20	-	15

Correlation		ODR *vs*						LDR *vs*				
P	O	16:0	18:0	18:1	18:2	18:3	LDR	16:0	18:0	18:1	18:2	18:3
+ve	+ve	-	-	-	1	19	1	-	-	-	-	14
+ve	-ve	-	1	-	1	-	-	-	-	-	-	-
+ve	ns	2	-	-	6	-	3	-	1	1	-	1
-ve	+ve	-	-	-	-	-	-	-	-	-	-	-
-ve	-ve	3	2	19	-	-	-	3	2	1	19	-
-ve	ns	2	3	-	-	-	1	2	1	2	-	-
ns	+ve	-	-	-	3	-	8	-	-	1	-	4
ns	-ve	5	6	-	2	-	1	6	5	7	-	-
ns	ns	7	7	-	6	-	5	8	10	7	-	-
Total	+ve	2	1	-	12	38	13	-	1	2	-	33
Total	-ve	13	14	38	3	-	2	14	10	11	38	-
Total	ns	23	23	-	23	-	23	24	27	25	-	5

concurrent selection for increased oil content and decreased linolenic acid content should be possible within both cultivars.

Although most correlations between individual fatty acids varied in direction, some trends were apparent. Palmitic acid was consistently positively correlated with stearic and negatively with linolenic, the last two fatty acids being usually negatively correlated. In all 19 lines, and in both generations, linolenic was strongly negatively correlated with oleic acid. The magnitude of this correlation was always greater than that of the negative correlation between linolenic and linoleic acid.

Variation within cv. Ward

Analysis of six individual seeds from each of four plants of the cv. Ward showed different degrees of within-plant variation in fatty acid composition. Two plants were relatively uniform, ranging from 33% to 44% and from 39% to 42% in linolenic acid content, whereas the other two plants showed considerable variation in this characteristic, ranging between 38% and 57%, and 18% and 50% respectively. This last, most variable plant was examined in greater detail by analysing 62 seeds using the half-seed technique. Linolenic acid content in these seeds varied between 22.4% and 49.7%, linoleic between 9.9% and 15.6%, and oleic between 29.4% and 59.0%. Palmitic and stearic acids did not vary substantially between seeds, averaging 5.4% and 2.8% respectively. Variation in linolenic acid content was strongly negatively correlated with that in oleic acid content ($r = -0.97$, $P < 0.01$) but was not correlated

with linoleic (0.20, n.s.).

Progeny plants were successfully established from 46 of the 62 half-seeds. Variation between their bulked 30-seed samples was considerably less than that between the parental half-seeds (Table 8). Oleic and linolenic acid contents in the progeny ranged from 17.6% to 28.6%, and 48.3% to 60.9%, respectively. This level of variation is approximately half the magnitude of that observed in the parental generation. The range of linoleic acid content in the progeny was similar to that in the parental generation. The tendency for mean performance of the offspring generation to be higher for linolenic acid content and lower for oleic acid content compared to the parental generation is probably related to their having been grown under different glasshouse environments.

Table 8: Fatty acid composition of parental seeds and offspring plants of the cv. Ward.

Generation	Fatty acid composition (%)					ODR	LDR
	16:0	18:0	18:1	18:2	18:3		
Parental (n = 62)							
Mean	5.4	2.8	40.8	12.4	38.7	1.31	3.15
s.d.	0.4	0.7	6.9	1.3	6.2	0.35	0.56
Offspring (n = 46)							
Mean	6.8	1.6	22.1	14.0	55.6	3.22	3.99
s.d.	0.2	0.2	2.8	0.9	2.9	0.55	0.38
Correlation (r)#	-0.07	-0.19	0.06	-0.12	-0.15	0.01	0.03

All correlation coefficients non-significant at the 5% level.

A parent-offspring correlation analysis revealed that there were no significant correlations between contents of any

of the fatty acids or ODR and LDR, indicating either that the large variation between parental seeds was not due to genetic differences or that there were substantial genotype x environment interactions between the two generations. The present study is unable to discriminate between these possibilities, either of which could explain the observation by Dorrell (1972) that selection for extreme fatty acid composition within this cultivar failed to achieve a consistent response.

2.4. DISCUSSION

Variation between Linum *species*

Seed oils of *Linum* species analysed in this study displayed wide variation in the relative proportions of the polyunsaturated fatty acids linoleic acid and linolenic acid, and for the presence of ricinoleic acid. By contrast, there was only minor variation in the proportions of the saturated palmitic and stearic acids, and the mono-unsaturated oleic acid.

With respect to linoleic and linolenic acids, two distinct fatty acid profiles were apparent and were associated with different taxonomic sections of the genus, *viz.* low-linoleic, high-linolenic species (Sections *Linum* and *Dasylinum*); and high-linoleic, low-linolenic species (Sections *Linastrum*, *Cathartolinum* and *Syllinum*). Similar patterns of variation have previously been observed by Plessers (1966) and Yermanos (1966). The large phenotypic differences between these distinct groupings (Figure 1), suggests that major gene differences exist

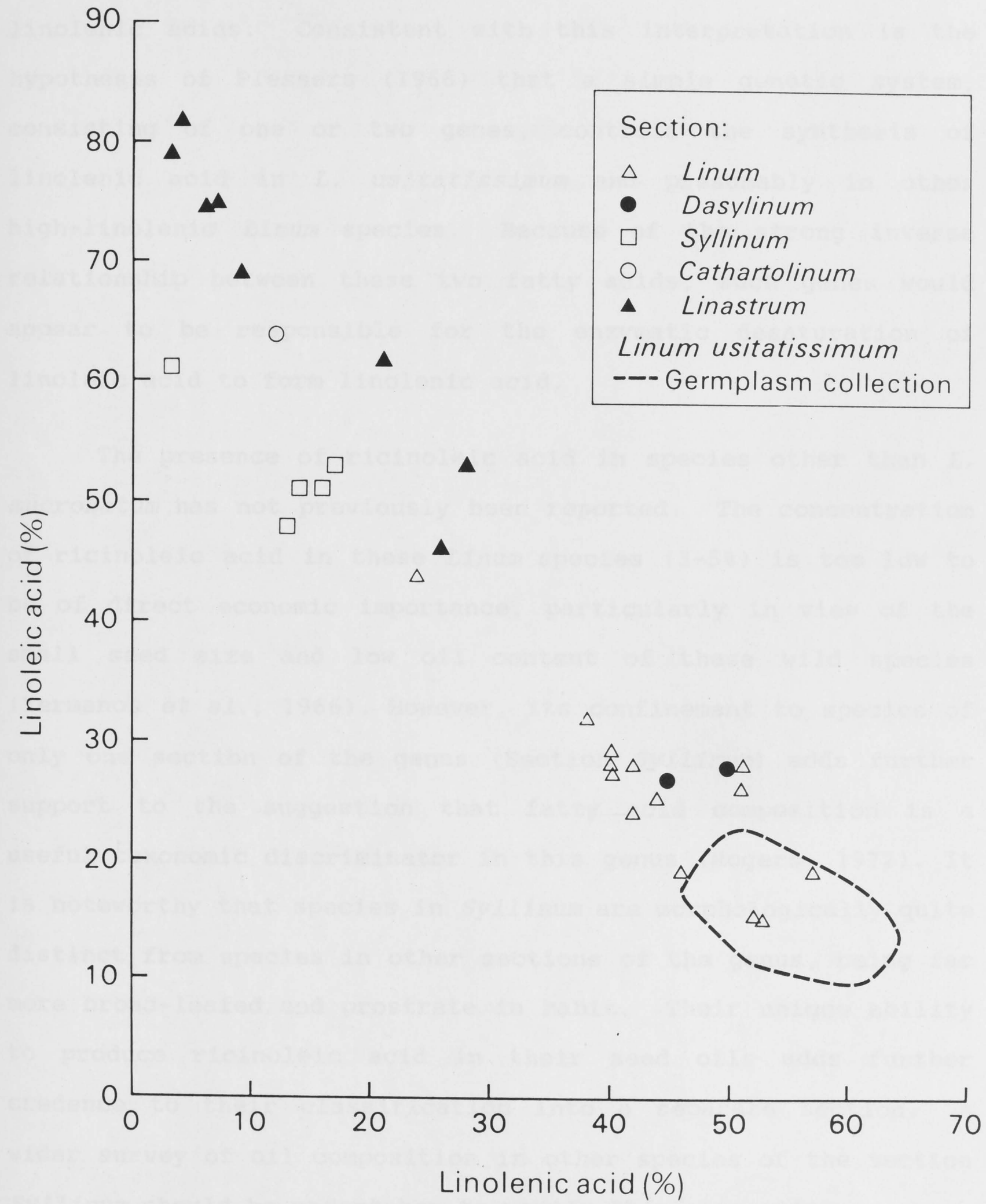

Figure 1: Pattern of variation of linoleic and linolenic acids in the species of the genus *Linum* classified according to section.

between *Linum* species influencing the synthesis of linoleic and linolenic acids. Consistent with this interpretation is the hypothesis of Plessers (1966) that a simple genetic system, consisting of one or two genes, controls the synthesis of linolenic acid in *L. usitatissimum* and presumably in other high-linolenic *Linum* species. Because of the strong inverse relationship between these two fatty acids, such genes would appear to be responsible for the enzymatic desaturation of linoleic acid to form linolenic acid.

The presence of ricinoleic acid in species other than *L. mucronatum* has not previously been reported. The concentration of ricinoleic acid in these *Linum* species (3-5%) is too low to be of direct economic importance, particularly in view of the small seed size and low oil content of these wild species (Yermanos *et al.*, 1966). However, its confinement to species of only one section of the genus (Section *Syllinum*) adds further support to the suggestion that fatty acid composition is a useful taxonomic discriminator in this genus (Rogers, 1972). It is noteworthy that species in *Syllinum* are morphologically quite distinct from species in other sections of the genus, being far more broad-leafed and prostrate in habit. Their unique ability to produce ricinoleic acid in their seed oils adds further credence to their classification into a separate section. A wider survey of oil composition in other species of the section *Syllinum* should be undertaken to verify this proposition.

The occurrence of high-linoleic low-linolenic species having similar levels of oleic acid to *L. usitatissimum* has important implications for the development of edible linseed

genotypes. Firstly, it suggests that linoleic acid desaturation might be genetically manipulated independently of oleic acid desaturation. Secondly, it suggests that there are unlikely to be any biological restrictions precluding the genetic reduction of linolenic acid in *L. usitatissimum*. However, the fact that no species was found that was devoid of linolenic acid suggests that complete removal of this fatty acid from linseed oil may not be possible.

Variation within Linum usitatissimum

In contrast to the extensive variation between *Linum* species in the relative proportions of linoleic and linolenic acids, variation within *L. usitatissimum* was considerably more limited. The germplasm collection analysed varied between 45% and 64% for linolenic acid and only between 10% and 18% for linoleic acid. These levels of linolenic acid are greater than for most other species in the section *Linum* (Figure 1), including *L. bienne* and *L. angustifolium*, considered to be the perennial and annual progenitors of *L. usitatissimum* respectively (Durrant, 1976). This increase in linolenic acid content presumably reflects selection for higher iodine value during the development of industrial quality linseed cultivars.

The level of variation in fatty acid composition in the germplasm collection analysed in this study is similar to that previously reported to exist in the World Collection of flax and linseed lines (Zimmerman and Klosterman, 1959). The presence of significant within-line variation was also confirmed in the

current study, and it was further demonstrated that, for each of the economically important characters, seed weight, oil content and fatty acid composition, at least some of the variation within several lines was due to genetic heterogeneity. The presence of such a level of heterogeneity is a reflection of the number of segregating breeding lines in the collection and also indicates that many commercial cultivars have been selected principally for yield and adaptability, rather than stringently for seed weight and oil characteristics. Prior to the extensive use of gas chromatography as an analytical technique, selection for oil composition was performed on the basis of iodine value, a measurement which can be the same in oils having different proportions of unsaturated fatty acids (Yermanos and Knowles, 1962). Thus cultivars selected for uniformity of iodine value could have remained heterogeneous for fatty acid composition.

A consequence of this genetic heterogeneity is that selection within lines should be practised before using them as parents in breeding programs. In this way plants may be chosen that have a more desirable level of expression than the line mean would suggest, rather than the less desirable level that could arise if random plants were chosen from a heterogeneous line. Dorrell (1972) adopted this rationale in selecting individual seeds having extreme fatty acid compositions from within the cultivar Ward. However the analysis of this variety in the present study indicated that, although considerable differences were evident between individual seeds (22-50% linolenic acid), such variation, in this instance, had no genetic basis, since their progeny all reverted to normal levels.

Selection for fatty acid composition in Linum usitatissimum

A principal objective of this study was to determine whether sufficient variation existed to reduce the content of linolenic acid in linseed oil by conventional hybridization and selection among existing germplasm. The results indicate that although significant variation exists for fatty acid composition, it is predominantly in the form of lines having higher levels of linolenic acid than Glenelg (*c.* 48%). The cultivar Avantgarde with 64% linolenic acid is a clear 7% higher than any other of the lines studied in detail, suggesting that a major gene may be responsible for this difference. From the germplasm collection it is possible to identify lines with contents of linolenic acid as low as 45%. If different genes control the low levels in these lines, a hybridization program aimed at recombining these genes could lead to further reduction. However, it seems unlikely that conventional selection within the available *L. usitatissimum* germplasm could reduce linolenic acid content to the very low level necessary to convert linseed into an edible oil.

Significant correlations between contents of individual fatty acids illustrate the likely correlated responses that would occur if selection was practised within *L. usitatissimum* for lowered linolenic acid content. Both oleic and linoleic acid contents were negatively correlated with linolenic. However, in none of the 19 lines studied in detail was the correlation between linolenic and linoleic greater than that between linolenic and oleic, in either the parental or offspring generation. This observation, combined with the same pattern in

the germplasm collection, indicates that selection for reduced linolenic acid content would tend to raise oleic to a greater degree than linoleic. Of interest in this regard is the cultivar Linfola 2 which has an above-average ODR value. Should low-linolenic, high-oleic genotypes be bred, this cultivar could be useful as a source of genes to raise the level of linoleic acid and hence the value of the product as an edible oil.

The tendency within *L. usitatissimum* for linolenic acid to be more negatively correlated with oleic than with linoleic acid contrasts with the strong negative relationship between linolenic and linoleic acids evident between *Linum* species. Additionally, the occurrence of such a consistently large negative correlation between oleic acid and linolenic acid is unexpected if, as is generally accepted, linolenic acid is produced by the sequential desaturation of oleic acid via linoleic acid through the action of substrate-specific desaturase enzymes. In this model a reduction in linolenic should result in a greater increase in the immediate precursor, linoleic, than in oleic acid. A possible explanation of these observations could be that variation between species is largely due to the presence of major gene effects on the desaturation of linoleic acid, whereas variation within *L. usitatissimum* is the result of many genes of smaller effect (Kenaschuk, 1975) that modify the desaturation of both oleic acid and linoleic acid either separately or together.

Use of wild Linum *species in breeding for low linolenic acid content in linseed*

Although it does not appear possible to eliminate

linolenic acid from linseed oil by selection within the natural variation in *L. usitatissimum*, the level of variation available within the wild *Linum* species appears sufficient to achieve this goal. The presence of ricinoleic acid in wild *Linum* species is of consequence to their utilization as a genetic resource in linseed breeding. Since ricinoleic acid would be an undesirable component in an edible oil, it would be necessary to select against its production in any hybrid involving a species from the section *Syllinum*. Thus it would appear preferable to concentrate such hybridization attempts on those low-linolenic acid *Linum* species not containing ricinoleic acid, that is, on species from the sections *Linastrum* and *Cathartolinum*.

A further factor potentially restricting the utilisation of wild *Linum* species as sources of low-linolenic genes is the postulated aneuploid origin of many species. Based on the frequency of species having various chromosome numbers, the basic chromosome number in the genus *Linum* is believed to be $x = 9$ (Harris, 1968; Durrant, 1976). Species having multiples of the basic number, such as $n = 2x = 18$ (e.g. *L. anglicum*) are considered to have a polyploid origin, with intermediate numbers, such as *L. usitatissimum* ($n = 15$), probably arising from the polyploid types by reduction in chromosome number. Wild species having low levels of linolenic acid could therefore have lost the capacity to synthesise this fatty acid either by inactivation of genes, through mutation, or by loss of the appropriate chromosome segment through either whole chromosome loss or as a consequence of chromosome rearrangement, such as Robertsonian fusion. In the latter case, a species lacking a segment that carries a linolenic acid synthesis gene may be

useful in deriving linseed genotypes that similarly lack this segment, through recombination in the interspecific hybrid. However if the lost segment also contains genes that are essential to the agronomic performance of linseed then this approach may not be of use. In view of this, and because there is currently no way of establishing the value of a species as a genetic resource, through determining the presence of structural genes controlling fatty acid biosynthesis, it would appear preferable to concentrate on the utilisation of the euploid (*n = 9,18*) low-linolenic wild species. In such species the reduced ability to synthesise linolenic acid is more likely to be caused by mutant alleles that might be able to be incorporated at homologous loci in linseed.

The successful utilisation of wild *Linum* species in linseed breeding would rely on obtaining appropriate fertile interspecific hybrids. Extensive studies on crossability between all the *Linum* species analysed for oil quality were performed during the course of this study. These investigations revealed that, with the exception of *L. bienne* and *L. angustifolium*, all species were reproductively isolated from *L. usitatissimum* (Green, unpublished data). It was shown that the nature of the crossability barrier varied between sections of the genus; species in sect. *Linum* were separated by post-fertilisation barriers that resulted in hybrid embryo degeneration, whereas species in other sections appeared to be more distantly related and were separated from *L. usitatissimum* by pre-fertilisation barriers such as failure of pollen germination or rejection of pollen tubes in the style. Although it may prove possible to obtain interspecific hybrids by embryo

culture within the sect. *Linum*, unfortunately those wild species are all high in linolenic acid. Crosses to suitable low-linolenic species (sects *Linastrum* and *Cathartolinum*) will require experimental techniques to be developed and applied and the prospects for utilisation of these species in linseed breeding is currently remote.

CHAPTER THREE

INDUCED VARIATION FOR SEED FATTY ACID COMPOSITION IN *L. USITATISSIMUM*.

3.1. INTRODUCTION

Surveys of seed fatty acid composition in *L. usitatissimum* revealed insufficient variation to enable the elimination of linolenic acid by selection (Chapter 2, and Zimmerman and Klosterman, 1959). Further, although the required genes appear to be present in related *Linum* species, they are currently unavailable for breeding, owing to the presence of strong interspecific crossing barriers. In these circumstances an alternative breeding strategy is to attempt to induce mutations in the genes controlling the synthesis of linolenic acid in the seed tissue of *L. usitatissimum*.

Mutation breeding has already proven successful in modifying the content of linolenic acid in rapeseed and soybean. Following treatment of rapeseed with ethyl methanesulphonate (EMS), Rakow (1973) reported the induction of a mutant (M57) in which linolenic acid content was reduced from 9.8% to 5.5%. Subsequent remutation of this line resulted in selections having as little as 3.2% linolenic acid (Robbelen and Nitsch, 1975). In soybean, a reduction in linolenic acid content of two percentage points was reported by Hammond *et al.* (1972) following X-ray mutagenesis. More recently, Wilcox *et al.* (1984) repeated this achievement by deriving a line having 3.4% linolenic acid following EMS treatment of the soybean cv. Century. Knowles (1983) has recommended the further application of this technique for genetic modification of fatty acid composition in oilseeds.

Several previous studies have demonstrated the potential

of mutagenesis for increasing variation for fatty acid composition in linseed. Rath and Scharf (1968) reported an increase in the range of refractive index, a measure of the degree of unsaturation of the oil, in the progeny of X-irradiated seeds. Similarly, gamma-ray treatment of seeds of the linseed variety NPRR9, which had an iodine value[1] of 144, increased the variability for this parameter, a range of 132 to 160 being observed in the M_4 generation (Srinivasachar and Malik, 1971). It was concluded that iodine value was particularly amenable to mutation breeding, perhaps because it was a simply inherited character. However, neither of these two studies was directed towards the isolation of low-linolenic acid genotypes; rather, the aim was to increase the level to improve the oil's drying quality.

More recently, the application of gas chromatography has permitted the detection of changes in individual fatty acids in mutagen-treated populations. Chemical mutagens have been shown to be effective in inducing variation in linolenic acid content in linseed in two separate experiments. Vereshchagin (1973) treated seeds with diethylsulphate and found M_4 seeds that had reduced levels of linolenic (49% reduced to 33%) and oleic acid (31% to 22%), and increased levels of linoleic (10% increased to 25%). Similar alterations to fatty acid composition were reported by Marquard *et al.* (1978) following treatment of linseed with EMS. Although there have been no reports of very

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1. Iodine value or number is a measurement of the degree of unsaturation of oil based on its capacity to accept iodine addition across carbon double bonds. The higher the iodine value, the more unsaturated is the oil.

low levels of linolenic acid, these studies clearly demonstrate that this character is amenable to manipulation through mutation breeding and suggest that low-linolenic genotypes might be developed, given adequate experimentation.

The availability of a rapid and sensitive chemical test for the presence of linolenic acid in vegetable oils (McGregor, 1974) enables large populations of seeds to be screened for induced mutations in much less time than by conventional chromatographic techniques. This assay, known as the TBA (thiobarbituric acid) test, relies on the formation of a red colour complex (Figure 2) when TBA reacts with the oxidation products of any fatty acid containing three conjugated double bonds (Dahle *et al.*, 1962). Since linolenic acid is the only such compound found in vegetable oils, the TBA test is specific for this fatty acid. Where linolenic acid is not present a yellow colour is observed, grading through orange to red as linolenic acid increases to 18% (McGregor, 1974). Because the content of linolenic acid in linseed is well above this level, and differences in intensity of the red colour are not as easy to see as are the colour changes at lower concentrations, this test will probably only be sensitive enough to detect reasonably large reductions in linolenic acid percentage in linseed. However, such reductions might be expected if the suggestion (Chapter 2 and Plessers, 1966) that linolenic acid content in *L. usitatissimum* is controlled by a small number of genes of major effect is correct.

This chapter reports the results of an induced mutation experiment in which the TBA test was used to detect linolenic

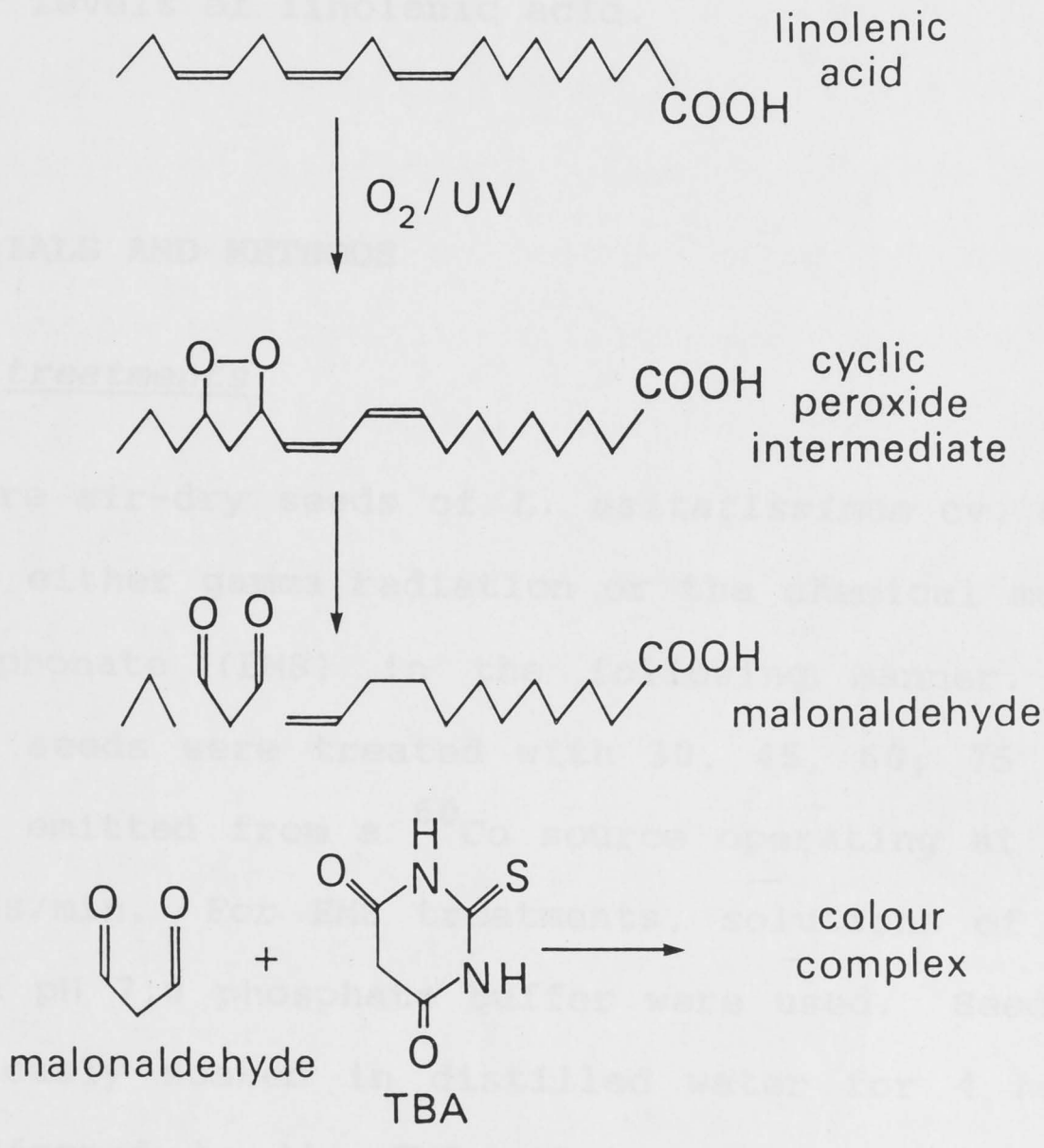


Figure 2: Chemical reactions involved in the TBA assay for linolenic acid content.

acid variants in mutagen-treated populations of the linseed cv. Glenelg. Two mutant lines, M1589 and M1722, each having reduced linolenic acid content and increased linoleic acid content, are described. The inheritance patterns of the induced mutations were examined in order to determine whether they were allelic or in different genes that might be recombined to give even lower levels of linolenic acid.

3.2. MATERIALS AND METHODS

Mutagenic treatments

Mature air-dry seeds of *L. usitatissimum* cv. Glenelg were exposed to either gamma radiation or the chemical mutagen ethyl methanesulphonate (EMS) in the following manner. For gamma radiation, seeds were treated with 30, 45, 60, 75 and 90 Krad gamma-rays emitted from a ^{60}Co source operating at a dose rate of 380 rads/min. For EMS treatments, solutions of either 0.3% or 0.4% in pH 7.0 phosphate buffer were used. Seeds which had been previously soaked in distilled water for 4 hours at $0^{o}C$ were transferred to the EMS solution and kept at $2^{o}C$ for 2 hours, then at $20^{o}C$ for 2 hours. Treated seeds were rinsed continuously in water for 16 hours at $2^{o}C$, then spread over nylon gauze and dried at room temperature. Immediately after drying, seeds were germinated in soil in paper tubes and subsequently transplanted to the field in widely-spaced rows at Ginninderra Experiment Station, Canberra, A.C.T., when approximately three weeks old.

Selection of linolenic acid mutants

At maturity 7072 M_1 plants were harvested and, where possible, a sample of 10 M_2 seeds taken for analysis from each plant. Because fatty acid composition in linseed is considered to be determined by the embryo genotype (Yermanos and Knowles, 1962) variants were selected by analysis of individual M_2 seeds. If an M_1 plant was heterozygous for a recessive induced mutant and was also non-chimeric, then on average one quarter of the M_2 seeds borne on that plant would be expected to be homozygous for the mutant allele and express the mutant phenotype. By sampling 10 M_2 seeds from such an M_1 plant, the probability of recovering at least one homozygous mutant genotype is 0.94 (Sedcole, 1977). Where induced mutants exhibit either additive or dominant gene action the probability of recovery is even higher.

Up to ten M_2 seeds from each M_1 plant were assayed non-destructively for linolenic acid in the following manner. Approximately one-quarter of the seed distal to the embryo was removed with a scalpel and the resulting seed 'chips' were arranged in a grid pattern on an adhesive acetate sheet to which a similar-sized sheet of Whatman No. 1 chromatography paper was then affixed. A second piece of chromatography paper was placed on top of the first and the 'sandwich' was passed through a pair of metal rollers to crush the seed chips and extrude the oil into the free piece of paper. The resulting test sheets, each containing oil spots from 96 seeds, were left overnight to allow even penetration of the oil into the paper fibres.

The test sheets were analysed the following day by a modification of the TBA method. Sheets were exposed to UV irradiation for 20 minutes in order to oxidize the linolenic acid, dipped in a 2% solution of thiobarbituric acid (TBA) and then lightly blotted. The TBA solution was freshly prepared by adding 1.8 g 2-thiobarbituric acid (4,6-dihydroxy-2-thiopyrimidine) to 60 ml of 50% v/v ethanol, heating to completely dissolve the TBA, and subsequently adding 30 mls of 20% w/v trichloroacetic acid. Colour development was achieved by clamping each individual test sheet between two glass plates and incubating in an oven at 110^{o}C for 20 mins. Mottling of the colour was prevented by placing additional sheets of paper between the glass plates and the test sheet. The spot colours were compared to those of a standard series of oils varying in their linolenic acid concentration, made by combining safflower oil (zero linolenic acid) and linseed oil (45% linolenic acid) in various proportions. Selections were made for low-linolenic acid amongst the M_2 seeds and selected plants grown by germinating the untested portion of the seed.

M_2, M_3, and M_4 progeny of the selections were grown under similar glasshouse conditions. Plants of cv. Glenelg that had not been mutagenically treated were grown as controls with each generation. Oil content and fatty acid composition were determined on samples of 30 seeds by the methods outlined in Chapter 1.3.

Genetic analysis of induced mutants

The parental lines used in this analysis consisted of the cultivar Glenelg and two M_4 plants derived from the induced

mutants M1589 and M1722, and apparently homozygous for the mutations. These plants were the progeny of the M_3 plants numbered M1589-8 and M1722-4 in Table 11 (page 64), but for convenience will be referred to simply as M1589 and M1722 in genetic studies. The crosses analysed were Glenelg x M1589, M1722 x Glenelg and M1722 x M1589. Single plants were used as parents in all crosses to produce F_1 and backcross populations. Additionally an F_2 population from the cross M1722 x M1589 was examined. Analysis of fatty acid composition was performed on a bulk sample of 30 seeds from each individual.

Individual crosses were grown in separate experiments as outlined below (population sizes shown in brackets):-

Experiment 1: *Glenelg x M1722*

Glenelg (5), M1722 (5), F_1 (4),
F_1 x Glenelg (10), F_1 x M1722 (15).
Planted October 1982, harvested March 1983.

Experiment 2: *M1589 x Glenelg*

Glenelg (6), M1589 (6), F_1 (6),
F_1 x Glenelg (20), F_1 x M1589 (20).
Planted December 1983, harvested May 1984.

Experiment 3: *M1722 x M1589*

M1722 (5), M1589 (5), F_1 (3),
F_1 x M1722 (27), F_1 x M1589 (27).
Planted September 1983, harvested March 1984.

Experiment 4: *M1722 x M1589*

F_2 (114)
Planted April 1983, harvested August 1983.

Means were calculated for all characters in the parental

and F_1 generations and compared using the *'t'* test. For characters that differed between the parents, the backcross generations were examined for segregation. Backcross plants were assigned to phenotypic classes based on the appearance of discontinuities at apparent break points in the frequency distributions. The proportions of plants observed in each phenotypic class were compared to those expected on the basis of appropriate genetic hypotheses. Goodness of fit to tested ratios was measured by the chi-squared method.

As several M1722 x M1589 F_2 plants had very low levels of linolenic acid (less than 2%) and were apparently homozygous for both the M1589 and M1722 mutant alleles, a further experiment was conducted to determine the fatty acid composition of all possible genotypes at the two loci. In this experiment (Experiment 5), Glenelg, M1589, M1722 and F_2 plant No. 69 (hereafter referred to as 'Zero') were crossed in a half-diallel design, that is, in all possible combinations excluding reciprocals. Parental and F_1 populations (6 plants per population) were grown together under the same glasshouse conditions as in Experiments 1 to 4 (planted April 1984, harvested September 1984). Chemical and statistical analyses were performed as for the previous experiments.

3.3. RESULTS

Variation in M_1 and M_2 generations

Considerable between-plant variation was observed for vigour and seed set in the M_1 population, with many plants

exhibiting total sterility. From a surviving population of 7072 fertile M_1 plants, 66,500 seeds were harvested, an average of 9.4 seeds per plant. This failure to achieve a sampling strategy of 10 seeds per plant was due to the occurrence of many plants that produced only 7, 8 or 9 seeds because of either partial sterility or adverse growing conditions.

Substantial variation in TBA colour was evident among the 66500 M_2 seeds analyzed. By comparison with the linolenic acid standards, 3840 M_2 seeds were judged to contain less than 40% linolenic acid, and 107 of these appeared to contain less than 30% linolenic acid. All mutagenic treatments gave rise to selections, EMS treatments resulting in a higher proportion of variants (Table 9). A total of 58 selections died following transplantation in the early stages of the experiment before the technique was refined. There was evidence that some M_2 seeds contained levels of linolenic acid greater than in cv. Glenelg, but no progeny of these seeds was tested.

Table 9: Frequency of selected M_2 seeds arising from various mutagenic treatments.

	EMS (%)		Gamma-rays (Krad)					Total
	0.3	0.4	30	45	60	75	90	
Tested	19235	21997	7649	2421	4411	5423	5364	66500
Selected	1257	1504	377	111	196	221	174	3840
Percent	6.5	6.8	4.9	4.6	4.4	4.1	3.2	5.8

Progeny testing of low-linolenic selections

When selections were progeny tested under glasshouse conditions, almost all of the surviving M_2 plants contained

normal levels of linolenic acid, that is, between 38% and 44%, indicating that the phenotypic variation observed in the M_2 seed population was largely due to environment. However two selections, M1589 and M1722, maintained reduced levels of linolenic acid, the M_3 seeds borne on both these M_2 plants containing 31.1% linolenic acid (Table 10). Both these selections also had elevated levels of linoleic acid and therefore lower linoleic desaturation ratios (LDR). The proportions of the other fatty acids and total oil content were within the range exhibited by the Glenelg control, but M1589 had smaller seeds. Although M1589 and M1722 were both derived from the 0.4% EMS treatment, they originated from different M_1 plants and hence from different treated seeds.

Table 10: Seed weight, oil content and fatty acid composition of Glenelg (mean of 9 plants) and M_2 single plants M1589 and M1722.

Line	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Glenelg	625	41.3	7.1	5.2	31.7	15.6	40.3	1.76	2.59
M1589	583	39.6	6.8	4.9	34.4	22.6	31.1	1.56	1.38
M1722	613	39.9	7.4	5.5	34.0	21.8	31.1	1.56	1.43

M1589 and M1722 were subsequently progeny tested by growing eight M_3 plants of each in the glasshouse, together with eight untreated Glenelg plants. On the basis of line mean performance, M1589 again had significantly smaller seeds than Glenelg but on this occasion also displayed significantly higher oil content (Table 11). M1722 did not differ from Glenelg in these characters. The proportions of palmitic, stearic and

Table 11: Seed weight, oil content and fatty acid composition of Glenelg and M_3 plants of M1589 and M1722.

	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	LDR and class #	
Glenelg									
1	660	42.9	7.1	4.3	37.1	14.0	37.4	2.67	high
2	680	40.0	7.1	4.4	39.7	13.5	35.1	2.60	high
3	623	39.0	7.2	4.2	34.0	15.1	39.4	2.61	high
4	645	40.9	7.3	4.5	36.3	15.0	36.8	2.45	high
5	652	42.5	7.3	4.5	34.6	15.3	38.1	2.49	high
6	628	41.4	7.4	5.2	38.9	13.7	34.4	2.51	high
7	670	41.0	7.4	4.8	36.8	14.2	36.6	2.58	high
8	635	40.6	7.4	4.6	38.2	13.9	35.7	2.57	high
Mean*	649[a]	41.0[a]	7.3[a]	4.6[a]	37.0[a]	14.3[a]	36.7[a]	2.56[a]	
M1589									
1	559	44.0	6.9	3.9	34.3	22.9	31.9	1.39	med
2	567	44.5	6.8	4.2	34.8	23.1	30.9	1.34	med
3	558	42.6	6.9	4.4	37.6	20.5	30.5	1.49	med
4	565	43.7	7.2	4.9	41.2	19.9	26.8	1.35	med
5	575	42.2	7.1	5.2	43.3	15.0	29.3	1.95	high
6	570	42.0	6.9	4.8	45.1	19.3	23.8	1.23	med
7	565	42.5	7.1	5.5	42.0	20.1	25.2	1.25	med
8	515	43.2	7.4	5.3	42.5	24.6	20.1	0.82	low
Mean	559[b]	43.1[b]	7.0[b]	4.8[a]	40.1[ab]	20.7[b]	27.3[b]	1.35[b]	
M1722									
1	620	39.1	7.5	4.6	36.5	15.2	36.0	2.37	high
2	640	41.2	7.3	4.2	39.2	18.1	31.0	1.71	med
3	615	41.8	7.0	4.0	38.4	19.2	31.3	1.63	med
4	660	37.9	7.4	4.6	40.8	24.8	22.3	0.90	low
5	605	39.7	7.4	4.3	41.2	19.8	27.2	1.37	med
6	638	39.7	7.6	4.5	39.6	19.2	29.1	1.52	med
7	605	41.4	8.2	4.7	41.5	24.4	21.0	0.86	low
8	642	38.5	7.8	5.4	40.6	17.9	28.2	1.58	med
Mean	628[a]	39.9[a]	7.5[a]	4.5[a]	39.7[b]	19.8[b]	28.3[b]	1.49[b]	

high: LDR > 1.9 medium: 1.0 < LDR < 1.9 low: LDR < 1.0

* Means in the same column having a superscript in common are not significantly different at the 5% level.

oleic acids were similar in all three lines. Both mutants again had significantly higher linoleic and lower linolenic acid, and hence lower LDR than Glenelg, in spite of considerable variation within both mutant lines. M1589 and M1722 each contained M_3 plants displaying the normal Glenelg fatty acid composition, as well as plants showing greater reductions in linolenic and greater increases in linoleic acid than were evident in the M_2 generation.

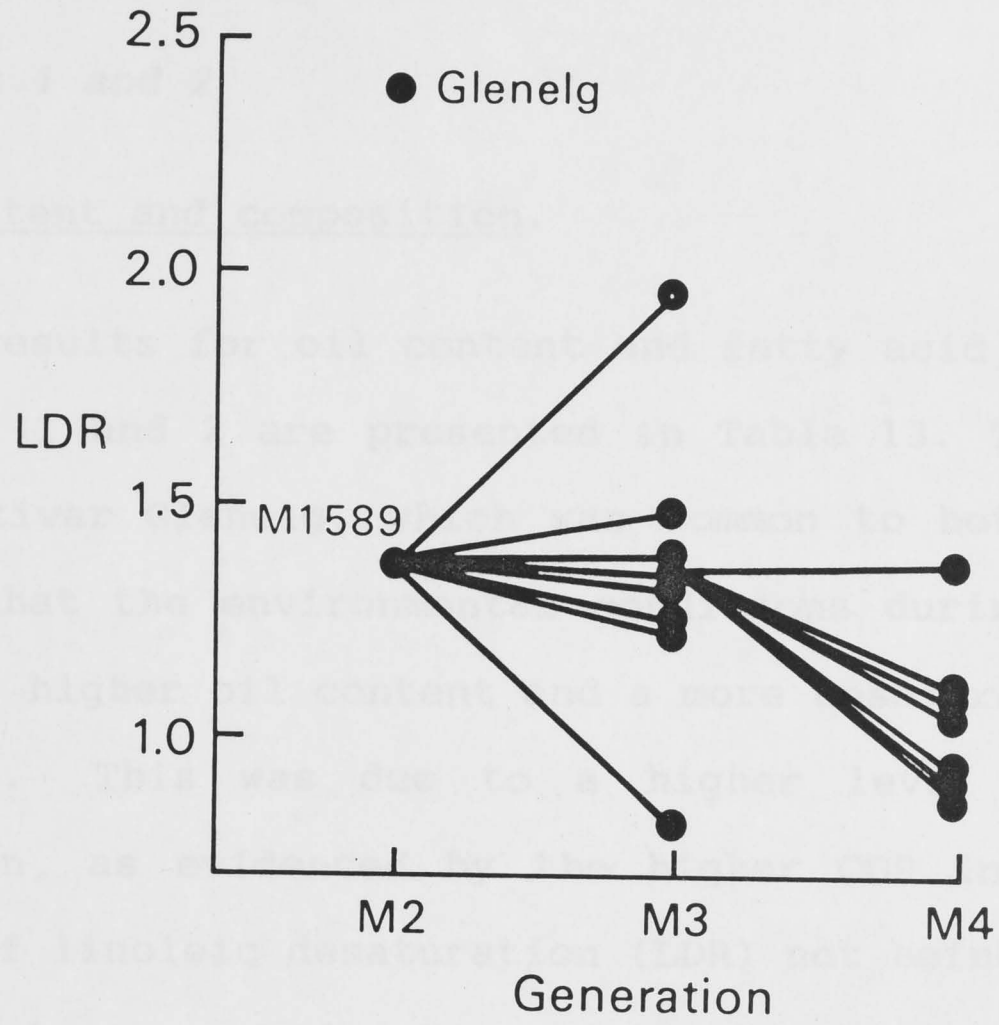
M_3 plants were classified as having either a high, medium or low LDR phenotype. The pattern of variation observed in these M_3 plants was consistent with that expected from the segregation of a single additive gene in which plants with a 'low' LDR are homozygous for the mutant allele and those with a 'medium' LDR are heterozygous. This was further supported by the performance of the M_4 generation in which six plants from an M1722 M_3 plant having a 'low' LDR maintained this phenotype, whereas seven M_4 plants from an M1589 M_3 plant having a 'medium' LDR showed segregation into 'low' and 'medium' LDR classes (Table 12, Figure 3). On the basis of this hypothesis the original two M_2 seeds selected by the TBA test would have been heterozygous for the mutant allele. Failure to recover the homozygous mutant genotypes from the sibling M_2 seeds of M1589 and M1722 was apparently due to either the absence of these genotypes in the samples (chance effects) or their loss during transplantation.

Table 12: Fatty acid composition of M_4 plants derived from M_3 single plant selections of M1589 and M1722.

M3 plant	M4 plant	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	LDR and class #
M1589-4	1	6.9	3.9	36.2	28.3	24.6	0.87 low
	2	7.5	4.2	28.2	29.3	30.7	1.05 med
	3	7.3	4.5	29.4	28.1	30.6	1.09 med
	4	6.8	3.8	36.9	27.6	24.8	0.90 low
	5	7.2	4.1	27.8	29.3	31.5	1.08 med
	6	7.0	5.2	33.4	28.7	25.6	0.89 low
	7	7.1	5.4	33.1	22.9	31.4	1.37 med
M1722-4	1	7.3	5.5	33.9	28.2	25.0	0.89 low
	2	7.2	4.7	36.4	26.7	24.9	0.93 low
	3	7.5	4.0	36.7	26.0	25.8	0.99 low
	4	7.3	4.0	32.5	27.5	27.6	1.00 low
	5	7.4	4.5	33.5	29.0	25.5	0.88 low
	6	7.5	4.6	36.3	26.8	24.7	0.92 low

high: LDR > 1.9 medium: 1.0 < LDR < 1.9 low: LDR < 1.0

In addition to mutants for fatty acid composition, altered phenotypes for other characters were observed in the M_1 and M_2 populations. These included increased plant height, albinism, yellow seed colour, blue flower colour, and various floral abnormalities such as reflexed petals.



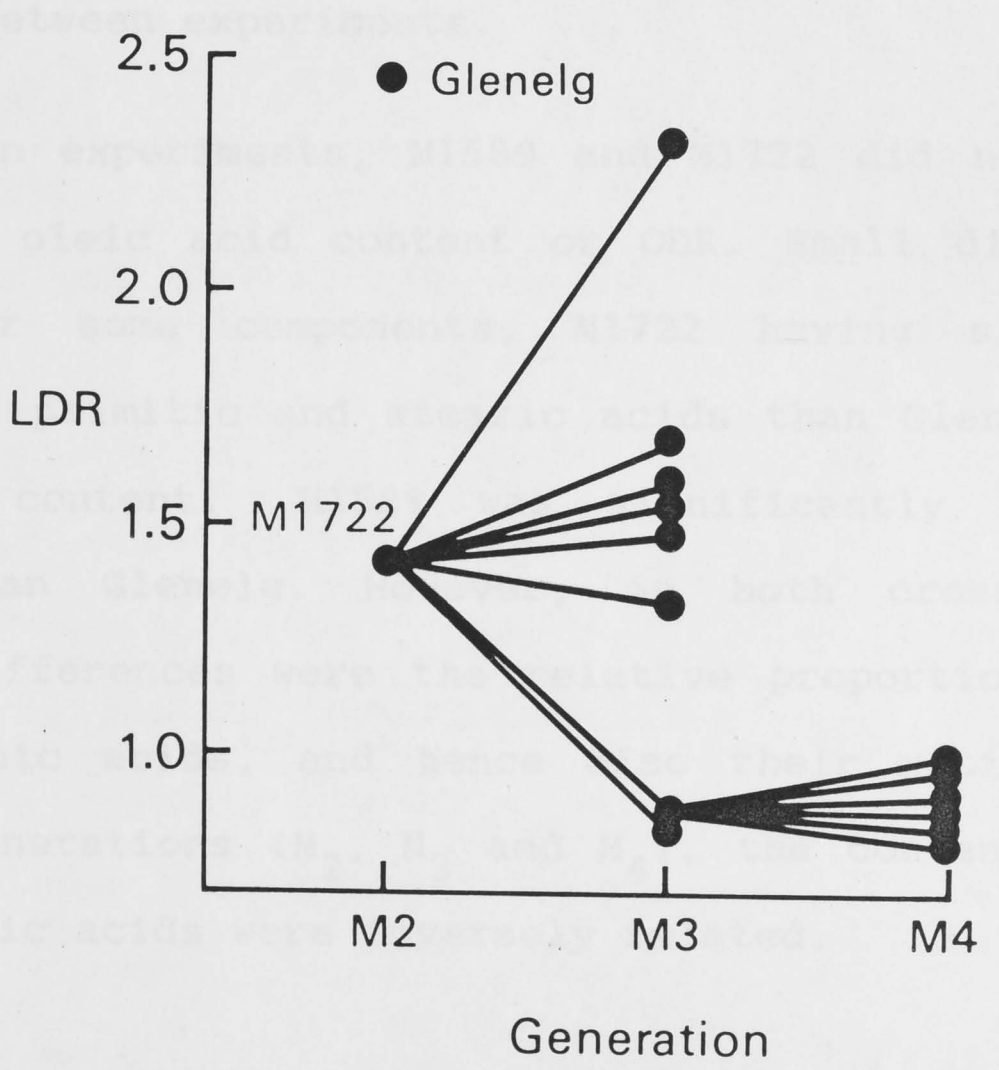


Figure 3: Pedigree and LDR value in M_2, M_3 and M_4 plants of M1589 and M1722.

Genetic analysis of M1589 and M1722 mutants

Experiments 1 and 2

(a) Oil content and composition.

The results for oil content and fatty acid composition in Experiments 1 and 2 are presented in Table 13. The performance of the cultivar Glenelg, which was common to both experiments, indicates that the environmental conditions during Experiment 1 resulted in higher oil content and a more unsaturated fatty acid composition. This was due to a higher level of oleic acid desaturation, as evidenced by the higher ODR in Experiment 1, the level of linoleic desaturation (LDR) not being significantly different between experiments.

Within experiments, M1589 and M1722 did not differ from Glenelg in oleic acid content or ODR. Small differences were evident for some components, M1722 having slightly higher contents of palmitic and stearic acids than Glenelg, and lower total oil content. M1589 was significantly higher in oil content than Glenelg. However, in both crosses the major parental differences were the relative proportions of linoleic and linolenic acids, and hence also their ratio, LDR. As in previous generations (M_2, M_3 and M_4), the contents of linoleic and linolenic acids were inversely related.

Table 13: Means and mid-parent values for oil content and fatty acid composition in the parental and F_1 generations of the crosses M1589 x Glenelg and Glenelg x M1722.

Genotype	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Experiment 1								
Glenelg	40.8^{a}	7.0^{a}	3.7^{a}	35.1^{a}	14.1^{a}	40.1^{a}	1.6^{a}	2.8^{a}
M1722	38.9^{b}	7.4^{b}	4.4^{b}	35.1^{a}	27.2^{b}	25.7^{b}	1.5^{a}	0.9^{b}
F_1	40.3^{ab}	7.4^{b}	4.2^{b}	35.7^{a}	20.9^{c}	31.7^{c}	1.5^{a}	1.5^{c}
MP value	39.9	7.2	4.1	-	20.7	32.9	-	1.9
Experiment 2								
Glenelg	36.9^{a}	8.1^{ab}	4.2^{a}	43.3^{a}	12.5^{a}	31.9^{a}	1.0^{a}	2.6^{a}
M1589	39.1^{b}	8.0^{a}	4.0^{a}	42.7^{a}	23.8^{b}	21.5^{b}	1.1^{a}	0.9^{b}
F_1	38.4^{b}	7.7^{b}	4.3^{a}	44.0^{a}	17.4^{c}	26.6^{c}	1.0^{a}	1.5^{c}
MP value	38.0	-	-	-	18.2	26.7	-	1.7

Within experiments means in the same column having a superscript in common are not significantly different at the 5% level.

In each cross the F_1 means for linoleic and linolenic acids and LDR were intermediate between those of the respective parental lines, and significantly different from both. The F_1 means for linoleic and linolenic acid in both crosses corresponded closely to the calculated mid-parent value, suggesting that co-dominant (additive) gene action was involved. However, in the case of LDR, both F_1 means deviated slightly from the mid-parent value in the direction of the mutant parent, suggesting that the mutant alleles may be partially dominant on this scale. In the presence of dominance, the deviation from the mid-parent value of a bulk seed sample

from a heterozygote will be less than the deviation of the heterozygous genotype itself. However the observed degree of dominance was minor relative to the magnitude of the gene effects, indicating that the use of bulk seed progeny to estimate the breeding value of a heterozygous genotype introduces little bias in the present case.

Single gene hypotheses were tested by comparing the observed segregations for LDR in the backcross generations with those expected for the segregation of co-dominant alleles at a single locus. Although bimodal frequency distributions were evident in the backcross generations for each of linoleic acid, linolenic acid and LDR, only the results for LDR are presented since discontinuities were clearer for this parameter (Figure 4). The clearer segregation pattern for LDR is probably due to the fact that it is a more accurate measure of linoleic desaturation activity than is either linoleic or linolenic acid percentage, the absolute values of which are also affected by variations in the level of oleic desaturation activity.

Based on the appearance of break points in the LDR frequency distributions (Figure 4), plants were assigned to three phenotypic classes, *viz.* 0.60 - 1.09, 1.10 - 1.99 and 2.00 - 2.99 (Table 14). The inferred genotypes of these classes are also shown in Table 14; the symbols *a* and *b* refer to the mutant alleles present in M1589 and M1722 respectively, *A* and *B* being the normal alleles present at these loci in Glenelg. Upper and lower case letters have been used only for clarity, not to indicate dominance. For both crosses the observed frequencies in the backcross generations do not differ significantly from

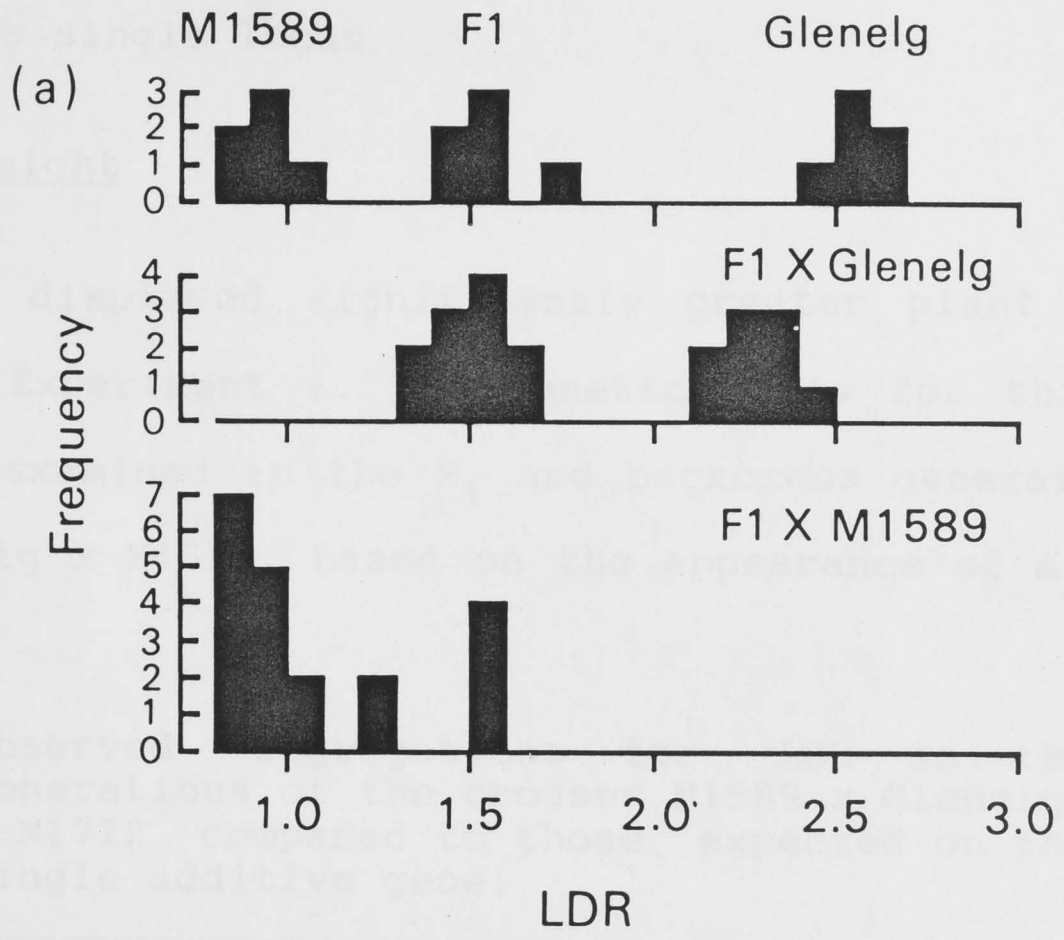


Figure 4: Frequency distribution for LDR in the parental, F_1 and backcross generations of the crosses (a) Glenelg x M1589 and (b) M1722 x Glenelg.

the 1:1 ratio expected for the segregation of co-dominant alleles at a single locus.

(b) Plant height

M1589 displayed significantly greater plant height than Glenelg in Experiment 1. The genetic basis for this increased height was examined in the F_1 and backcross generations of the cross Glenelg x M1589. Based on the appearance of a break-point

Table 14: Observed segregations for LDR in the backcross generations of the crosses M1589 x Glenelg and Glenelg x M1722 compared to those expected on the basis of a single additive gene.

LDR class and inferred genotypic constitution	Observed frequency#	
Experiment 1		
	F_1 x M1722	*F_1 x Glenelg*
0.60 - 1.09 (*bb*)	9 (7.5)	-
1.10 - 1.99 (*Bb*)	6 (7.5)	6 (5)
2.00 - 2.99 (*BB*)	-	4 (5)
Total	15 (15)	10 (10)
Chi-squared (1 df)	0.60 ns	0.40 ns
Experiment 2		
	F_1 x M1589	*F_1 x Glenelg*
0.60 - 1.09 (*aa*)	14 (10)	-
1.10 - 1.99 (*Aa*)	6 (10)	11 (10)
2.00 - 2.99 (*AA*)	-	9 (10)
Total	20 (20)	20 (20)
Chi-squared (1 df)	3.20 ns	0.20 ns

Expected frequencies in brackets

at 120 cm in the frequency distributions for these generations (Figure 5), individuals were classified as being either short or tall. In the F_1 generation plant height was identical to that of Glenelg, indicating that shortness was dominant over tallness. The backcross to Glenelg contained only short plants, again indicating dominance, whereas the backcross to M1589 segregated 11 short : 9 tall. This ratio is not significantly different from the 1:1 ratio expected for the segregation of a single dominant gene for shortness (X^2 = 0.20, P > 0.65).

(c) Seed weight

Seed weight of M1722 was similar to that of Glenelg but that of M1589 was significantly lower, showing an approximately 17% reduction in Experiment 1. This is similar to the 14% reduction observed in the M_3 generation. The M1589 x Glenelg F_1, and the backcross to Glenelg, had normal sized seeds, that is, not significantly different from Glenelg's, indicating that large-seededness is dominant over small-seededness. The backcross to M1589 displayed a complete range in seed weight and a break-point was assigned at 490 mg, resulting in a segregation of 12 small : 8 normal for seed weight. This ratio agrees with the 1:1 expected for the segregation of a single dominant gene for large seed size (X^2 = 0.80, P > 0.4).

(d) Joint segregation

In order to determine whether the recessive mutants for increased plant height and reduced seed weight were associated with the LDR mutant, either by pleiotropy or linkage, the backcross generation (M1589 x Glenelg) x M1589 was classified

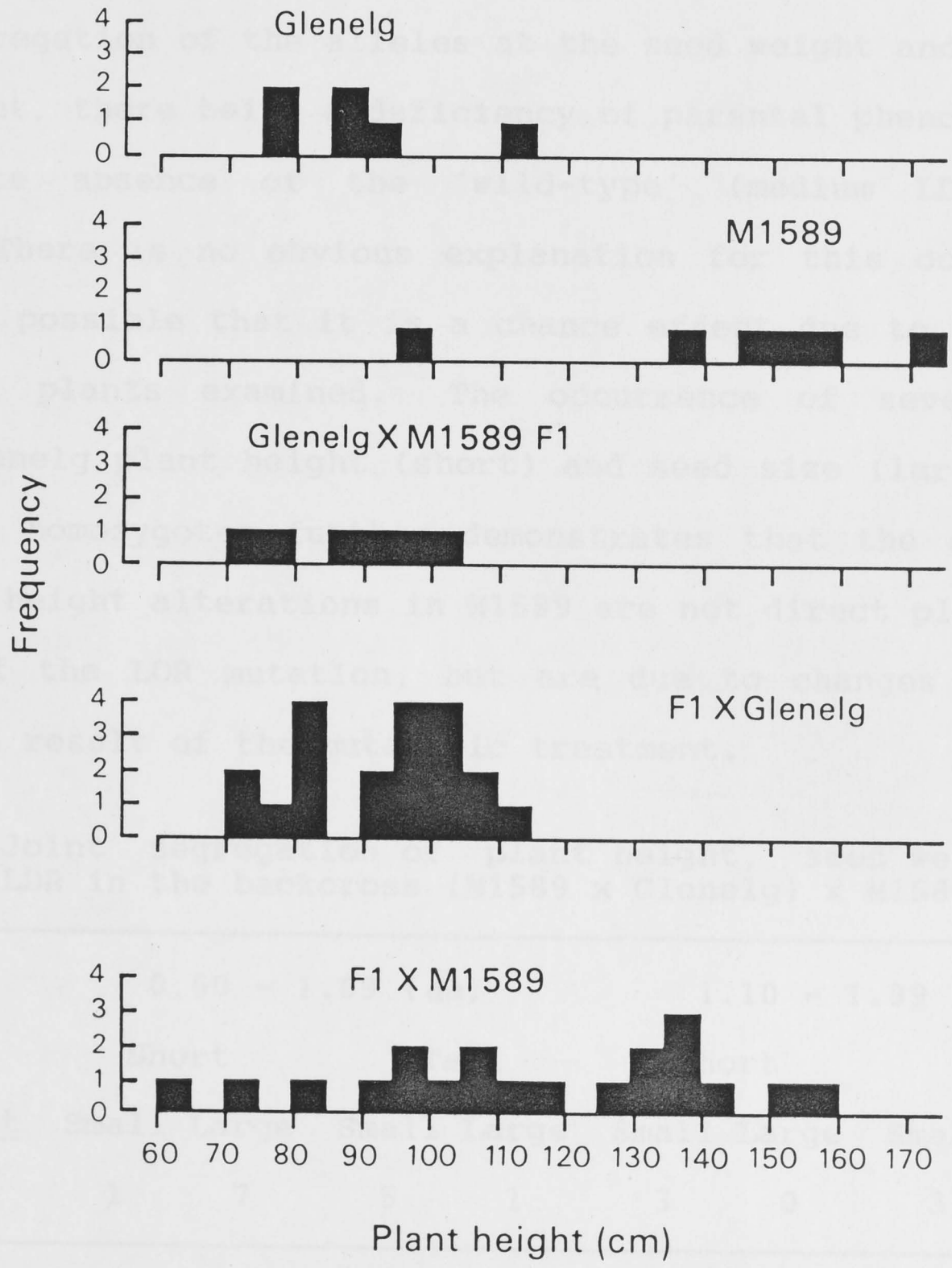


Figure 5: Frequency distribution for mature plant height in the parental, F_1 and backcross generations of the cross Glenelg x M1589.

for phenotypes of all three characters and the chi-squared test applied to pairwise combinations of loci (Table 15). The data indicate that the locus controlling seed weight variation in M1589 is linked to the loci controlling plant height and LDR, but no linkage was detected between these latter two loci. The joint segregation of the alleles at the seed weight and LDR loci is aberrent, there being a deficiency of parental phenotypes and a complete absence of the 'wild-type' (medium LDR, large seeds). There is no obvious explanation for this occurrence, but it is possible that it is a chance effect due to the small number of plants examined. The occurrence of seven plants having Glenelg plant height (short) and seed size (large) among the 14 *aa* homozygotes further demonstrates that the seed size and plant height alterations in M1589 are not direct pleiotropic effects of the LDR mutation, but are due to changes to other genes as a result of the mutagenic treatment.

Table 15: Joint segregation of plant height, seed weight and LDR in the backcross (M1589 x Glenelg) x M1589.

LDR	0.60 - 1.09 (*aa*)				1.10 - 1.99 (*Aa*)			
Height	Short		Tall		Short		Tall	
Seed weight	Small	Large	Small	Large	Small	Large	Small	Large
Obs. freq.	1	7	5	1	3	0	3	0

Single locus segregations (tested against 1:1 ratio)

LDR X^2 = 3.20ns Height X^2 = 0.20ns Seedweight X^2 = 0.80ns

Joint segregations (test of independence)

LDR-Seed weight: X^2 = 5.71sig.(5%) LDR-Height: X^2 = 0.09ns

Seed weight - Height: X^2 = 5.69 sig.(5%)

Experiments 3 and 4

In order to determine whether the induced LDR mutants present in M1589 and M1722 are at the same or different loci, the cross M1722 x M1589 was analysed. Fatty acid composition data for all plants analysed in this cross are presented in Appendix 2. In Experiment 3, M1589 had significantly higher oil content, higher oleic and lower palmitic, linoleic and linolenic acid contents than M1722. Thus ODR was lower in M1589 than in M1722, but LDR was identical. The F_1 was similar to M1589 in oil and palmitic acid contents, and to M1722 in content of other fatty acids and ODR value. The F_1 generation was not significantly different from either parent for LDR (Table 16).

Table 16: Mean oil content and fatty acid composition in the parental and F_1 generations of the cross M1722 x M1589.

Genotype	Oil (%)	Fatty acid composition (%)					ODR	LDR
		16:0	18:0	18:1	18:2	18:3		
M1722	38.1^{a}	8.4^{a}	4.8^{a}	35.4^{a}	27.8^{a}	23.3^{a}	1.5^{a}	0.8^{a}
M1589	40.4^{b}	7.2^{b}	5.0^{a}	44.0^{b}	24.5^{b}	19.1^{b}	1.0^{b}	0.8^{a}
F_1	41.9^{b}	7.4^{b}	4.9^{a}	37.6^{a}	27.0^{ab}	22.8^{a}	1.3^{a}	0.9^{a}

Means in the same column having a superscript in common are not significantly different at the 5% level.

If M1722 and M1589 carry identical mutations in the same gene affecting LDR, then the F_1 genotype will be homozygous for this mutation and hence genetically identical to both parents, and the backcross and F_2 generations will not show genetic segregation. On the other hand, if the mutations are at different loci, the M1722 x M1589 F_1 will be heterozygous at both loci and the backcross and F_2 generations will show

segregation, the proportions of various genotypic classes being dependent on the degree of linkage between the two loci.

The frequency distributions for LDR in all generations of the cross M1722 x M1589 are shown in Figure 6. Segregation in the F_2 and backcross generations is present, with five classes readily apparent. The upper three classes correspond to those present in the Glenelg x M1589 and Glenelg x M1722 crosses, whereas the two additional classes having lower LDR are novel. These classes suggest the hypothesis that the M1589 and M1722 mutations are in different genes, the lower LDR phenotypes arising by recombination of mutant alleles. Under this hypothesis the F_2 plants having an LDR of between 0.20 and 0.59 would be homozygous for the mutant at one locus and heterozygous at the other, whereas the plants in the lowest LDR class would be homozygous for the mutant alleles at both loci.

The observed LDR class frequencies are consistent with the segregation pattern expected for a hypothesis of two independently segregating genes acting additively to control the relative proportions of linoleic and linolenic acids (Table 17). Among the seven F_2 plants in the lowest LDR class, linoleic and linolenic acids averaged 50.6% and 1.6% respectively. This fatty acid composition phenotype is hereafter referred to as 'zero'-linolenic. All seven 'zero'-linolenic F_2 plants were progeny tested by growing ten F_3 plants, all of which had the same fatty acid composition as their F_2 parents, confirming the homozygosity of mutant alleles at both loci in this phenotypic class.

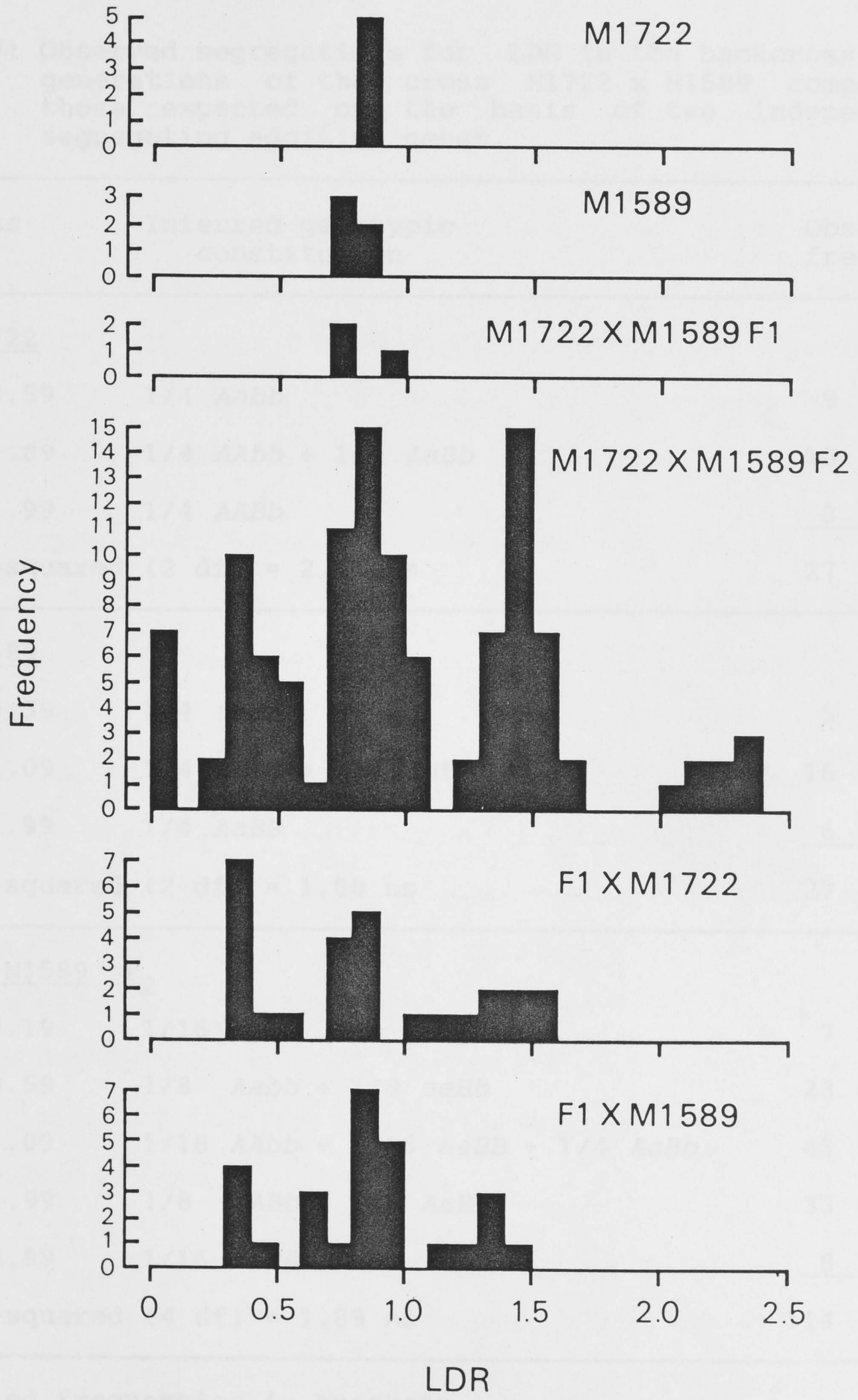


Figure 6: Frequency distribution for LDR in the parental, F_1, F_2 and backcross generations of the cross M1722 x M1589.

Table 17: Observed segregations for LDR in the backcross and F_2 generations of the cross M1722 x M1589 compared to those expected on the basis of two independently segregating additive genes.

LDR class	Inferred genotypic constitution	Observed frequency[#]
$\underline{F_1 \text{ x M1722}}$		
0.20 - 0.59	1/4 *Aabb*	9 (6.75)
0.60 - 1.09	1/4 *AAbb* + 1/4 *AaBb*	10 (13.5)
1.10 - 1.99	1/4 *AABb*	8 (6.75)
Chi-squared (2 df) = 2.41 ns		27 (27)
$\underline{F_1 \text{ x M1589}}$		
0.20 - 0.59	1/4 *aaBb*	5 (6.75)
0.60 - 1.09	1/4 *aaBB* + 1/4 *AaBb*	16 (13.5)
1.10 - 1.99	1/4 *AaBB*	6 (6.75)
Chi-squared (2 df) = 1.00 ns		27 (27)
$\underline{\text{M1722 x M1589 } F_2}$		
0.00 - 0.19	1/16 *aabb*	7 (7.1)
0.20 - 0.59	1/8 *Aabb* + 1/8 *aaBb*	23 (28.5)
0.60 - 1.09	1/16 *AAbb* + 1/16 *aaBB* + 1/4 *AaBb*	43 (42.5)
1.10 - 1.99	1/8 *AABb* + 1/8 *AaBB*	33 (28.5)
2.00 - 2.99	1/16 *AABB*	8 (7.1)
Chi-squared (4 df) = 1.89 ns		114 (114)

[#] Expected frequencies in brackets

Experiment 5

All possible combinations of genotypes at both mutant loci, produced by crossing Glenelg, M1589, M1722 and 'Zero' in a half diallel design, were analysed for fatty acid composition (Table 18). No substantial variation was present between genotypes for contents of palmitic, stearic or oleic acids, or for ODR value. However considerable variation was observed for linoleic and linolenic acids (Figure 7), which were completely negatively correlated (r = -0.98), and hence also for LDR.

Genotypes were grouped according to their number of mutant alleles at the two loci (Table 18). As expected, significant between-class differences were observed for linoleic acid, linolenic acid and LDR. LDR value declined progressively as the number of mutant alleles increased, but this reduction was not completely linear, with decrements of decreasing magnitude as the number of mutant alleles increased. Although this departure from complete additivity is only small, it is consistent with similar observations in the M1589 x Glenelg and Glenelg x M1722 crosses and therefore supports the previous suggestion that the mutant alleles may be partially dominant over the normal alleles when analysed on the LDR scale.

Within the classes having 0, 1, 3 or 4 mutant alleles, the contents of linoleic and linolenic acids, and the value of LDR, were similar, illustrating that allelic substitutions at either locus have equal effect. However, within the class having 2 mutant alleles, LDR was slightly, but significantly, higher in the *AaBb* genotype than in the *AAbb* and *aaBB* genotypes. This

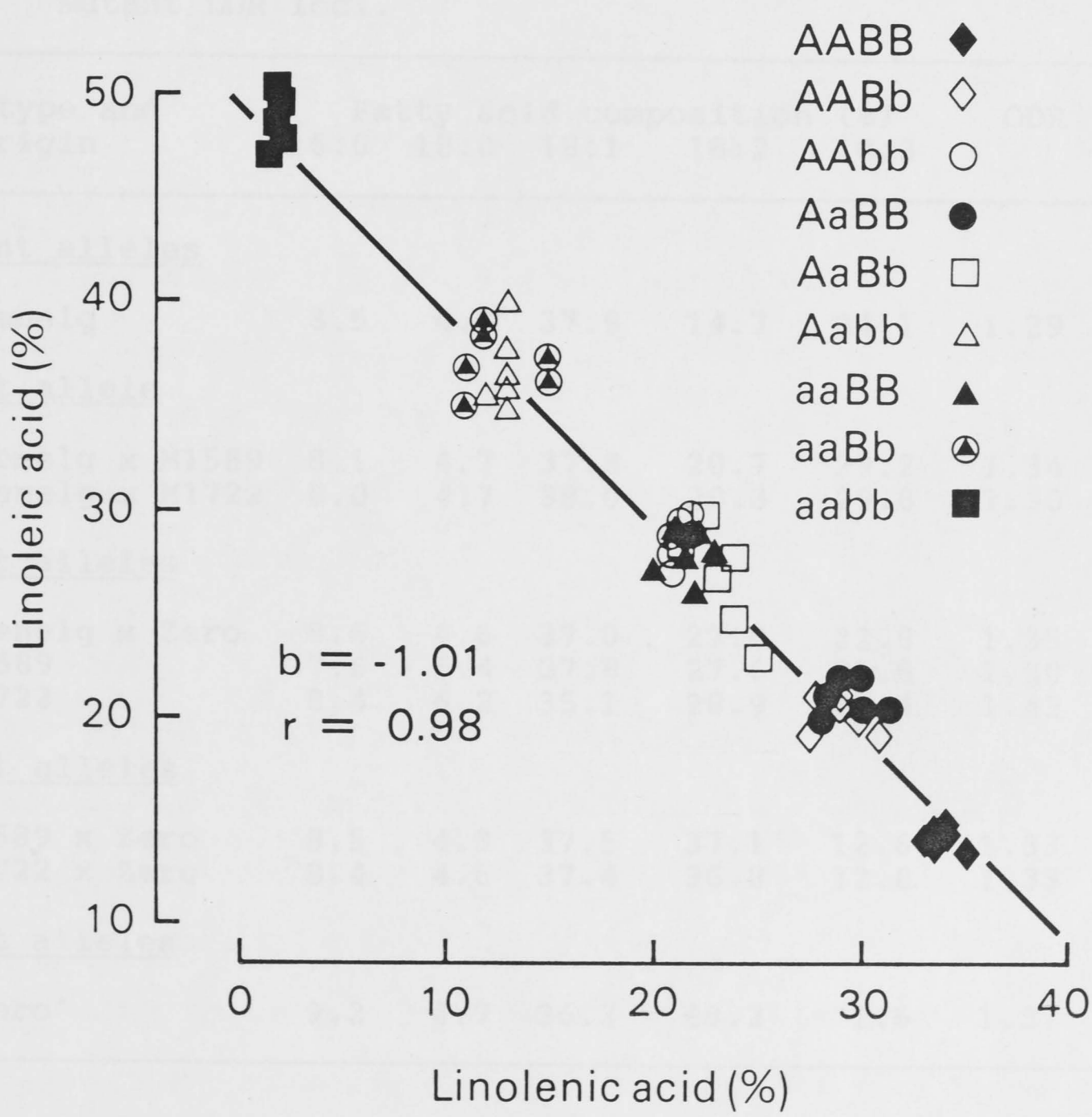


Figure 7: Relationship between linoleic and linolenic acid contents in all possible genotypes at the M1589 and M1722 mutant loci.

seems to indicate that the wild-type alleles are slightly dominant over the mutant alleles, in contrast to the indication of partially dominant mutant alleles when the single gene inheritances were examined on the LDR scale.

Table 18: Fatty acid composition of all genotypes at the two mutant LDR loci.

Genotype and origin	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
No mutant alleles							
AABB Glenelg	8.5	4.8	37.9	14.7	34.1	1.29	2.32
1 mutant allele							
AaBB Glenelg x M1589	8.1	4.7	37.3	20.7	29.2	1.34	1.42
AABb Glenelg x M1722	8.0	4.7	38.0	20.3	29.0	1.30	1.43
2 mutant alleles							
AaBb Glenelg x Zero	8.6	4.6	37.0	27.0	22.8	1.35	0.84
aaBB M1589	7.6	5.4	37.8	27.6	21.6	1.30	0.78
AAbb M1722	8.4	6.2	35.1	28.9	21.4	1.43	0.74
3 mutant alleles							
aaBb M1589 x Zero	8.5	4.3	37.5	37.1	12.6	1.33	0.34
Aabb M1722 x Zero	8.4	4.6	37.4	36.8	12.8	1.33	0.35
4 mutant alleles							
aabb 'Zero'	9.2	4.7	36.3	48.2	1.6	1.37	0.03

3.4. DISCUSSION

Induced variation for fatty acid composition

Although variation for linolenic acid content in the M_2 seed population was great, only two mutant lines were eventually recovered from a selected population of 3782 seeds. The

reversion to normal phenotype by the progeny of almost all of the selections indicates that the variation for linolenic acid content in the M_2 seed population was predominantly due to environmental rather than genetic causes. During the seed maturation period in the field, maximum daily temperature rose above 25°C on 39 days and above 30°C on 13 of these days. Canvin (1965) demonstrated that increasing the temperature from 21°C to 26.5°C reduced the level of linolenic acid from 53% to 38% in the linseed cv. Redwood. In the present study (see Chapter 4), when the cv. Glenelg was experimentally subjected to a similar temperature increase, linolenic acid content was reduced from 42% to 36%. Thus the occurrence of reduced linolenic acid content in a large proportion of the M_2 seed population was not unexpected.

In those M_3 and M_4 plants apparently homozygous for the M1589 and M1722 mutants, the average concentration of linoleic and linolenic acids were 26.9% and 24.3% respectively. This represents a reduction in the linoleic desaturation ratio from 2.58 in the parental cultivar Glenelg to 0.90. These values transgress the range of fatty acid composition observed in the *L. usitatissimum* germplasm collection (Chapter 2), in which the highest linoleic acid content was 20.9% and the lowest linolenic acid content was 45.5%. The combination of the M1589 and M1722 mutants into a single genotype resulted in even further reduction in linolenic and increase in linoleic acid content, proportions of other fatty acids remaining unaltered. The high levels of linoleic and low levels of linolenic in these recombinant genotypes were similar to the extreme levels observed amongst wild *Linum* species (Chapter 2), and confirm the

suggestion, made on the basis of fatty acid patterns in wild *Linum* species, that oleic acid desaturation and linoleic acid desaturation can be manipulated independently.

Although the possibility of producing mutant lines having altered proportions of linoleic and linolenic but with no changes in the proportions of other fatty acids was suggested by the wild *Linum* species data, their occurrence was somewhat unexpected in view of the previously observed interrelationships between fatty acids in *L. usitatissimum*. In a collection of 1175 flax and linseed varieties, Zimmerman and Klosterman (1959) observed a complete negative correlation between variety means of linolenic and oleic acids, with no correlation between linolenic and linoleic acids. In the present study, although a significant negative correlation involving linolenic and linoleic acid was observed among *L. usitatissimum* germplasm (Chapter 2), it was concluded that variation in linolenic acid content was principally related to that in oleic acid. Yermanos and Knowles (1962) in reciprocal crosses between cultivars differing in fatty acid composition found no evidence of concurrent variation between linolenic and linoleic acids. Similarly, when Canvin (1965) substantially reduced the level of linolenic acid in cv. Redwood by growing it at high temperatures, there was an equivalent increase in oleic, with relatively minor alteration to the content of linoleic acid. It is also noteworthy that the unstable lines having 13% linolenic acid selected from within the cv. Ward also had 79% oleic acid (Downey and Dorrell, 1971). The strong relationship between linolenic acid and linoleic acid present in the mutant lines, and the corresponding absence of an association with oleic acid,

is therefore novel within *L. usitatissimum*.

Genetic control of linoleic and linolenic acid biosynthesis

The mutants induced in this study indicate that in *L. usitatissimum* cv. Glenelg the desaturation of linoleic acid to linolenic acid can be controlled by two independently-segregating major genes acting additively, each 'wild-type' allele at either locus being responsible for an increase of approximately ten percentage points in linolenic acid content. Such major gene effects for fatty acid composition have not previously been reported in linseed. Das and Rai (1974) concluded that in a diallel involving five linseed parents, iodine value was controlled by a number of minor genes acting additively. More recently, Doucet and Filipescu (1981) analysed a diallel involving three linseed and four flax varieties, and found no evidence of major gene action controlling the proportions of unsaturated fatty acids. Likewise, genes of minor effect have generally been considered to be responsible for the limited variation observed in linolenic acid content in other oilseed species such as rapeseed (Kondra and Thomas, 1975) and soybean (White *et al.*, 1961; Martin *et al.*, 1983). Thus, the relatively small differences in linolenic acid content that exist between Glenelg and the majority of current linseed varieties are presumably due to the cumulative effects of several minor genes that modify the expression of the two major genes identified in the present study.

However, some varieties of linseed have much higher levels of linolenic acid than does cv. Glenelg. For example cv. Avantgarde has 64% linolenic acid, a clear 7% higher than

the other 18 varieties examined in detail in Chapter 2, and 19% higher than cv. Glenelg. It may be that a third major gene is responsible for this large increment in linolenic acid content. Alternatively, Avantgarde may carry different alleles at the two loci already identified that result in greater linoleic acid desaturation activity than those present in Glenelg. Further genetic studies are necessary to resolve this point.

Although no variants for oleic desaturation were selected from the present study, the possibility that major genes control the desaturation of oleic acid to linoleic acid in *L. usitatissimum* is suggested by studies involving other crop species. In oilseeds such as safflower, sunflower and maize, which contain little or no linolenic acid, the wide ranges of linoleic : oleic ratios (i.e. ODR) have been demonstrated to be determined by major genes. Normally safflower has a high linoleic (75 - 80%) and low oleic acid content (10 - 15%), but types having low linoleic (12 - 30%) and high oleic (64 - 83%) have been identified (Horowitz and Winter, 1957; Knowles and Mutwakil, 1963). Further, an intermediate type having approximately equal proportions of oleic and linoleic acids has been reported (Knowles and Hill, 1964). These differences in composition were demonstrated to be under the control of a single genetic locus with three alleles *Ol*, ol^1 and *ol*. Similar variations in oleic and linoleic acids have been identified in sunflower, also normally a high-linoleic, low-oleic oil (Downey and Dorrell, 1971). In maize, the 20% difference in oleic and linoleic acids between related lines R84 and IHO is controlled by a single gene, the *In* locus, high oleic acid content being dominant over low (Poneleit and Alexander, 1965; de la Roche *et*

al., 1971). Thus, as is the case for linoleic desaturation, the desaturation of oleic acid appears to be under simple genetic control, suggesting that it might be possible to inactivate this step by induced mutation breeding, in order to produce high-oleic, low-linoleic linseed genotypes should they become commercially desirable. Smaller modifications should be achievable by selection within the existing ODR variation reported in Chapter 2.

Proposed gene symbols

For clarity during presentation of results the symbols *a* and *b* have been used to represent the mutant alleles controlling LDR in M1589 and M1722 respectively. The use of upper and lower case symbols normally indicates dominance, and, because these genes have now been shown to exhibit additive (codominant) gene action, it is necessary to assign more appropriate symbols. It would also be desirable to choose symbols that are indicative of the functions of the genes; thus, because both Linoleic and Linolenic acids are altered equally by the alleles at these two loci, the gene symbols *Ln1* (M1589) and *Ln2* (M1722) are proposed. The *Ln* symbol has been previously used to designate differences in linoleic acid content in maize (de la Roche *et al.*, 1971). Alleles in the mutant lines should be designated by the superscript 0, with those present in cv. Glenelg being assigned the superscript 1. Any subsequently identified allelic variants would be classified by successive numerals. Under this system the homozygous genotypes in this study would be designated as follows:-

Glenelg	$Ln1^1Ln1^1$ $Ln2^1Ln2^1$
M1589	$Ln1^0Ln1^0$ $Ln2^1Ln2^1$
M1722	$Ln1^1Ln1^1$ $Ln2^0Ln2^0$
'Zero'	$Ln1^0Ln1^0$ $Ln2^0Ln2^0$

These symbols are used for the remainder of this dissertation.

Conversion of linseed oil into an edible oil

The mutants induced in this study provide the germplasm required to convert linseed into an edible oil seed. At less than 2%, the level of linolenic acid in the recombined mutants is below that of commercial cultivars and currently available breeding lines of both soybean and rapeseed. Additionally, the large increase in linoleic acid content has resulted in an oil which is more polyunsaturated than either soybean or rapeseed, the composition being similar to that of high-linoleic maize oil. Thus, provided the reduction in linolenic acid has been sufficient to prevent flavour reversion, low-linolenic linseed should be suitable for use as an edible oil. Further increases in linoleic acid content might be achieved by either of two methods. Firstly, lines in which the desaturation of oleic to linoleic acid is increased might be selected from crosses involving 'Zero' and genotypes identified in Chapter 2 as having a larger ODR. Secondly, increased linoleic acid content in 'Zero' might result from cultivation at lower temperatures than used in this study. The degree of unsaturation of several oilseeds, including linseed, is known to be increased by low temperatures. In the present study 'Zero' had 48.3% linoleic acid when grown at 27/20^{o}C, which is above the field

temperatures normally experienced by maturing linseed crops. Linoleic acid of field-grown 'Zero' might therefore be reasonably expected to be higher and might approach the 62% level required for the production of polyunsaturated margarines. This prospect is fully examined in a Phytotron study reported in the following chapter.

The 'Zero' genotype, being derived by induced mutagenesis and recombination from the adapted commercial cultivar Glenelg, may be directly useful as a cultivar. Their appear to be no undesirable pleiotropic effects of the $Ln1^0$ and $Ln2^0$ alleles, but one or two generations of backcrossing to Glenelg are desirable in order to select against the height and seed weight mutations originating from M1589 and any other, still-unidentified background mutations that may have resulted from the EMS treatment.

Conversion of other linseed cultivars to low linolenic acid content should be readily achieved by routine backcrossing procedures. The additive gene action at the *Ln1* and *Ln2* loci, combined with the large phenotypic differences associated with the mutant alleles, means that backcross plants heterozygous at both loci can be identified by the fatty acid composition of their selfed seed, without the need to progeny test. This is demonstrated by the plot of linoleic acid against linolenic acid in the Glenelg x Zero cross (Figure 7) where the $Ln1^1Ln1^0$ $Ln2^1Ln2^0$ class is clearly distinct from both the $Ln1^1Ln1^0$ $Ln2^1Ln2^1$ and $Ln1^1Ln1^1$ $Ln2^1Ln2^0$ classes expected to occur in the (Glenelg x 'Zero') x Glenelg backcross generation. The efficiency of backcross breeding would be further improved if

double heterozygotes can be identified at the embryo rather than mature plant stage. This would be possible if the fatty acid composition of the embryo is determined by its own genotype and not by that of the maternal parent on which it is borne. The presence of maternal effects for fatty acid composition is examined in Chapter 5.

CHAPTER FOUR

EFFECT OF TEMPERATURE ON SEED FATTY ACID COMPOSITION IN *L. USITATISSIMUM*

4.1. INTRODUCTION

When grown under a post-flowering temperature regime of 27°C (day)/20°C(night) in previous experiments, the seed oil of the 'zero'-linolenic genotype consisted predominantly of linoleic acid (48%) and oleic acid (36%), together with the saturated fatty acids, palmitic (9%) and stearic acid (5%). It therefore approached the composition of sunflower, a premium quality edible oil. Since the minimum industry requirements for the manufacture of 2:1 and 3:1 polyunsaturated margarines are 62% and 72% linoleic acid respectively, a further increase in linoleic acid content would be necessary before the 'zero'-linolenic linseed oil could be used extensively in such products. Harris *et al.* (1978) demonstrated that the content of linoleic acid in sunflower seed oil was substantially increased by low temperatures during seed maturation. If 'zero'-linolenic linseed genotypes show a similar temperature response, it is possible that field grown plants will produce acceptable levels of linoleic acid (> 62%), since linseed is grown as a winter crop and usually matures under relatively cool conditions in southern Australia.

The effect of temperature on the fatty acid composition of traditional high-linolenic linseed genotypes is well documented. Yermanos and Goodin (1965) found that increases in post-flowering temperature drastically reduced linolenic acid content and increased oleic, linoleic remaining constant. Pre-flowering temperature treatments, although having significant effects on vegetative development, did not influence the fatty acid composition of the seed oil. Similarly, Canvin

(1965) observed that when post-flowering temperature was increased from 10°C to 26°C linolenic acid declined from 64% to 40%, and linoleic from 18% to 11%, while oleic increased from 11% to 41% in the cultivar Redwood. By altering day and night temperature independently, Dybing and Zimmerman (1965) demonstrated that both high day temperatures and high night temperatures caused a reduction in the degree of unsaturation of the seed oil. Likewise, Beringer and Saxena (1968) observed lower levels of unsaturated fatty acids when day temperature was 28°C compared to 12°C, night temperature being kept constant at 12°C.

Thus, in all of these studies involving high-linolenic linseed genotypes, there was a higher degree of unsaturation of the oil when plants matured at low temperatures. It was therefore of interest to determine whether the fatty acid composition of the novel 'zero'-linolenic linseed genotype had a similar temperature response, as well as to compare the thermal stability of its desaturase enzyme systems with that of its parental variety, Glenelg, and the individual mutant genotypes from which it was derived. This chapter outlines the results of such a study in which Glenelg, M1589, M1722 and 'Zero' were grown under a range of post-flowering temperature treatments in a controlled environment facility.

4.2. MATERIALS AND METHODS

The four linseed genotypes examined in this study were cv. Glenelg, M1589, M1722 and 'Zero'. M1589 and M1722 were

derived from Glenelg by induced mutation, each being homozygous for a different single-gene mutation causing reduced levels of linolenic acid in the seed oil (see Chapter 3). 'Zero' was derived from the cross M1722 x M1589, is homozygous for both induced mutations and is almost devoid of linolenic acid. With the exception of the two linolenic acid mutations and any other mutations that may have occured during the initial EMS treatment, these four lines are isogenic.

Plants were grown in 15 cm pots of vermiculite/ perlite (1:1) and watered twice daily, in the morning with Hoagland's nutrient solution and in the afternoon with tap water. Photoperiod control consisted of natural daylength supplemented by incandescent lights to provide a 16 hr day/8 hr night regime throughout the experiment. All plants were grown in one glasshouse at 24/19^{o}C in the CSIRO Phytotron (Canberra, Australia) until flowering, at which time three plants (replicates) of each genotype were randomly allocated to each of six glasshouses under the following day/night temperature regimes:- 15/10^{o}C, 18/13^{o}C, 21/16^{o}C, 24/19^{o}C, 27/22^{o}C and 30/25^{o}C. Thermoperiod control consisted of 8 hr day temperature and 16 hr night temperature.

Plants were harvested at maturity and analysed by the methods outlined in Chapter 1.3. Due to poor seed yield at 30/25^{o}C, insufficient seed was available to determine seed weight and oil content accurately, but fatty acid composition was determined on a 10-seed sample.

Data for seed weight, oil content, palmitic acid, stearic acid, oleic acid and ODR were analysed by analysis of variance

techniques, in which the error component comprised the pooled effects of replicates and its interactions with genotypes, temperatures and genotype x temperature interaction. Because of large between-genotype differences in the magnitude of linoleic acid content, linolenic acid content and LDR, and the corresponding large differences in between-plant variability, separate analyses of variance were performed for individual genotypes or groups of genotypes for these characters. For both linoleic and linolenic acid this involved two analyses, one for 'Zero' and one for the remaining three genotypes combined. For LDR, separate analyses were performed for both Glenelg and 'Zero', and a combined analysis for M1589 and M1722. Stearic acid content was analysed on arcsine transformed data. Genotype/temperature combination means were compared using the least significant difference calculated from the residual mean square.

4.3. RESULTS

Genotype means (of the three replicate plants) for all characters at each temperature are presented in Table 19. Analyses of variance revealed highly significant genotype and temperature effects for all characters, and significant genotype x temperature interaction for all characters except seed weight and oleic acid content. However these interactions were minor when compared to the main effects of genotype and temperature and no major reversals of genotype ranking occurred between temperature treatments.

Table 19: Seed weight, oil content and fatty acid composition of the linseed genotypes Glenelg, M1589, M1722 and Zero grown under a range of post-flowering temperature regimes.

Temp. (°C)	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Glenelg									
15/10	927	44.1	5.8	3.2	18.8	21.7	49.9	3.82	2.30
18/13	849	43.1	6.0	3.4	26.3	18.8	45.4	2.44	2.42
21/16	783	43.3	6.5	3.9	31.5	15.5	42.2	1.84	2.73
24/19	614	41.3	7.3	6.3	31.0	15.1	40.0	1.80	2.65
27/22	630	39.5	8.0	6.5	35.0	14.6	35.7	1.44	2.45
30/25	-	-	8.6	7.8	33.5	15.7	34.0	1.49	2.16
l.s.d.	26	1.6	0.4	0.5	1.6	1.5	2.4	0.33	0.14
M1589									
15/10	777	33.6	6.6	4.4	20.7	34.9	33.2	3.30	0.95
18/13	742	35.4	6.4	3.5	27.7	34.0	28.2	2.25	0.83
21/16	668	34.8	6.8	4.4	33.7	29.4	25.6	1.65	0.87
24/19	558	34.3	7.4	6.4	35.2	27.5	23.3	1.45	0.86
27/22	505	33.6	7.5	6.5	38.7	24.8	21.9	1.22	0.88
30/25	-	-	8.9	8.4	36.2	24.2	21.9	1.27	0.90
l.s.d.	26	1.6	0.4	0.5	1.6	1.5	2.4	0.33	0.08
M1722									
15/10	874	34.9	6.2	4.0	23.9	33.1	32.6	2.76	0.96
18/13	840	36.8	6.2	3.9	31.5	30.0	28.3	1.86	0.94
21/16	779	34.7	6.9	4.7	35.8	26.6	26.0	1.47	0.98
24/19	650	34.8	8.0	6.2	35.4	25.9	24.3	1.42	0.94
27/22	585	32.2	8.9	6.6	37.8	25.6	20.8	1.23	0.81
30/25	-	-	9.2	8.9	35.0	29.3	17.2	1.33	0.59
l.s.d.	26	1.6	0.4	0.5	1.6	1.5	2.4	0.33	0.08
'Zero'									
15/10	867	42.0	6.2	3.2	17.3	70.2	3.1	4.24	0.04
18/13	826	40.0	6.4	3.3	25.1	62.7	2.3	2.61	0.04
21/16	753	39.9	7.4	4.4	29.0	57.3	1.8	2.04	0.03
24/19	649	40.7	8.1	6.0	28.0	55.9	1.8	2.07	0.03
27/22	592	38.1	8.5	6.3	32.1	50.9	1.9	1.65	0.04
30/25	-	-	9.5	7.2	33.7	46.9	2.5	1.47	0.05
l.s.d.	26	1.6	0.4	0.5	1.6	3.3	0.3	0.33	0.01

l.s.d. = Least significant difference at the 5% level.

Within each temperature treatment, oil content for M1589 and M1722, and seed weight for M1589, were significantly lower than for the other genotypes. Substantial genotypic differences for linoleic acid, linolenic acid and LDR were evident at all temperatures, both M1589 and M1722 being intermediate between Glenelg and 'Zero' for these characters. Although statistically significant at most temperatures, the differences between M1589 and M1722 were small compared with their differences from Glenelg and 'Zero'. Between-genotype differences for palmitic acid, stearic acid, oleic acid and ODR were minor and statistical significance was attained at only some temperatures.

Plants of the same genotype exposed to different temperature treatments showed significant differences for most characters analysed. When randomly assigned to temperature treatments at the commencement of flowering, plants of the same genotype were of approximately the same size, but their subsequent growth was strongly influenced by temperature. Plants placed at 15/10^{o}C and 18/13^{o}C continued to grow and flowered profusely, producing high yields of large seeds. Plants placed at higher temperatures than these did not show substantial further growth, flowered for a shorter period, and produced lower yields of significantly smaller seeds. At 30/25^{o}C, flowers were poorly developed and capsules were small, containing a high proportion of aborted and shrivelled seed. In spite of these considerable effects, oil content of fully-developed seeds was similar within genotypes at all temperatures up to 24/19^{o}C, declining slightly at 27/22^{o}C. As noted in Section 4.2., oil content was not determined at

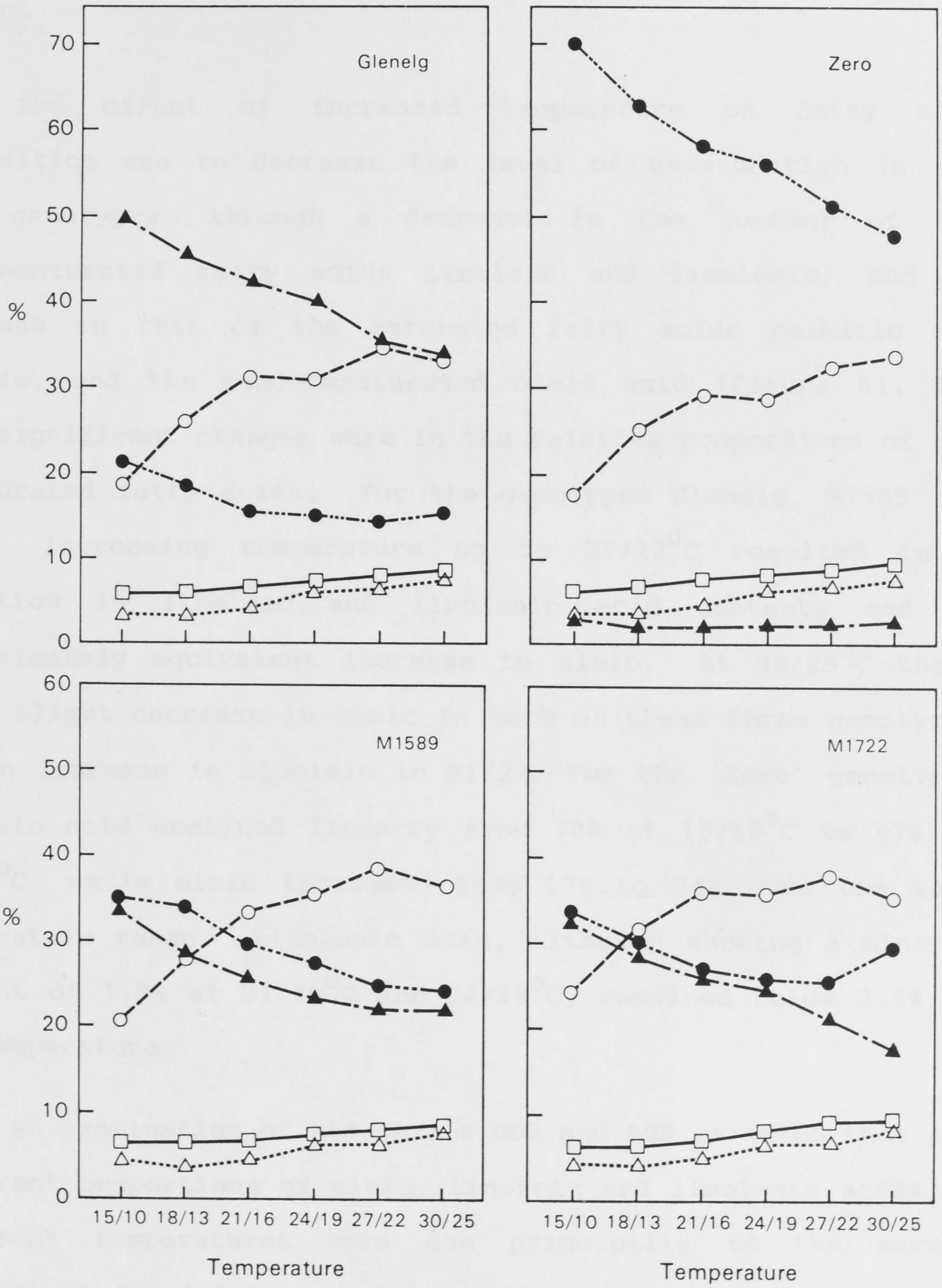


Figure 8: Relationship between post-flowering temperature and proportions of palmitic (□——□), stearic (△·····△), oleic (○– – –○), linoleic (●—···—●) and linolenic (▲—·—▲) acids in the seed oil of cv. Glenelg and the mutant genotypes M1589, M1722 and 'Zero'.

30/25°C.

The effect of increased temperature on fatty acid composition was to decrease the level of unsaturation in all four genotypes, through a decrease in the content of the polyunsaturated fatty acids linoleic and linolenic; and an increase in that of the saturated fatty acids palmitic and stearic, and the mono-unsaturated oleic acid (Figure 8). The most significant changes were in the relative proportions of the unsaturated fatty acids. For the genotypes Glenelg, M1589 and M1722, increasing temperature up to 27/22°C resulted in a reduction in linoleic and linolenic acid contents and an approximately equivalent increase in oleic. At 30/25°C there was a slight decrease in oleic in each of these three genotypes and an increase in linoleic in M1722. For the 'Zero' genotype, linoleic acid declined linearly from 70% at 15/10°C to 47% at 30/25°C, while oleic increased from 17% to 34% over the same temperature range. Linolenic acid, although showing a minimum content of 1.8% at 21/16°C and 24/19°C, remained below 3.1% at all temperatures.

An examination of the ratios ODR and LDR revealed that the different proportions of oleic, linoleic and linolenic acids at different temperatures were due principally to the marked temperature sensitivity of the initial enzymatic conversion of oleic acid to linoleic acid. For all four genotypes, an increase in temperature from 15/10°C to 21/16°C resulted in approximately a 50% reduction in the value of ODR, with further, but smaller, reductions at higher temperatures (Figure 9). By contrast, LDR was remarkably insensitive to temperature

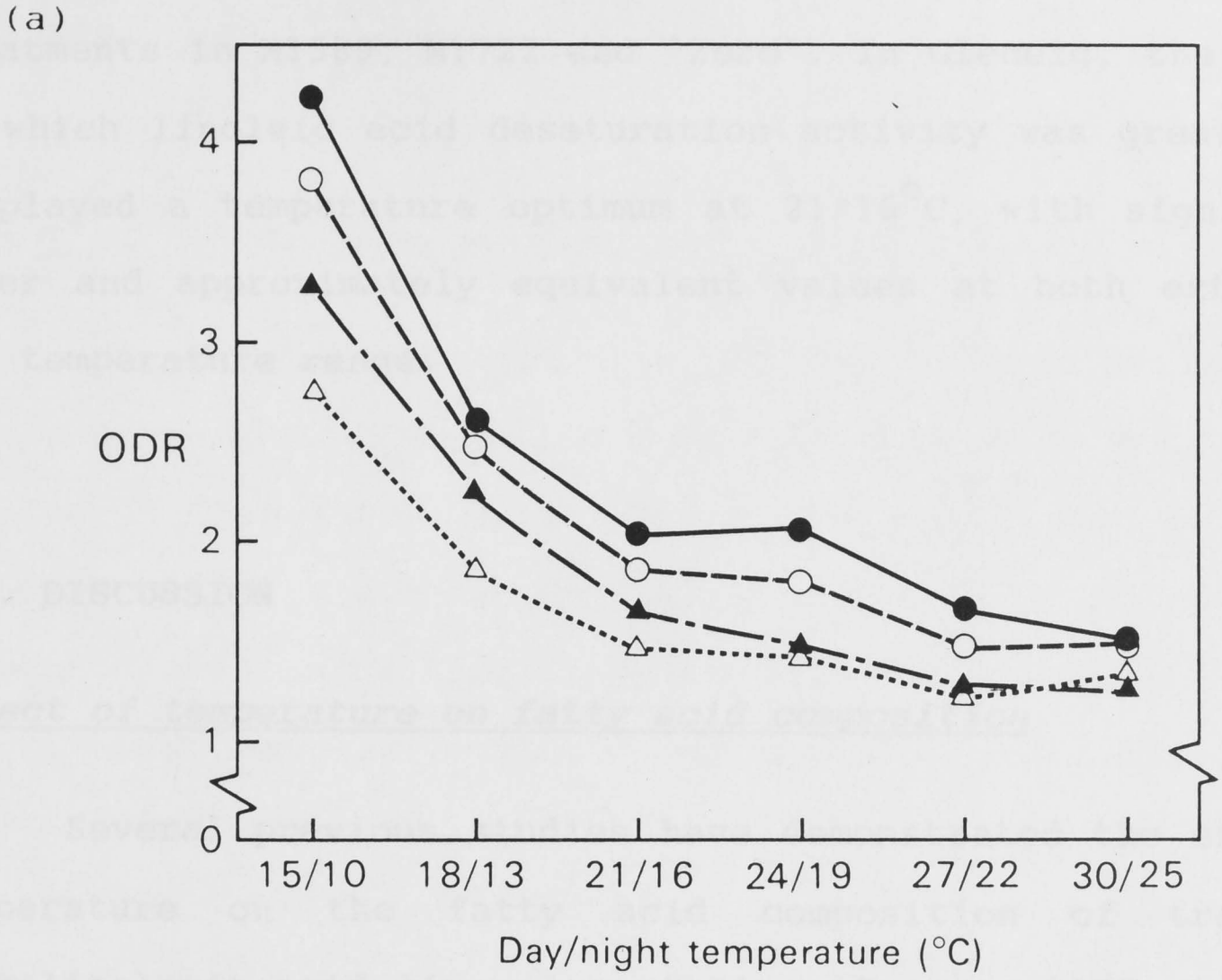


Figure 9: Relationship between post-flowering temperature and the values of (a) ODR and (b) LDR in the seed oil of of cv. Glenelg (○--○) and the mutant genotypes M1589 (▲-·-▲), M1722 (△·····△) and 'Zero' (●——●).

treatments in M1589, M1722 and 'Zero'. In Glenelg, the genotype in which linoleic acid desaturation activity was greatest, LDR displayed a temperature optimum at 21/16^{o}C, with significantly lower and approximately equivalent values at both extremes of the temperature range.

4.4. DISCUSSION

Effect of temperature on fatty acid composition

Several previous studies have demonstrated the effects of temperature on the fatty acid composition of traditional high-linolenic acid linseed varieties (Canvin, 1965; Dybing and Zimmerman, 1965; Yermanos and Goodin, 1965). The performance of the high-linolenic cultivar Glenelg in the present study is consistent with these previous reports. High temperatures during the seed maturation period have pronounced effects on the relative proportions of the unsaturated fatty acids; linoleic and linolenic acids are considerably lowered and there is an equivalent increase in the content of oleic acid. Palmitic and stearic acids both increase slightly but significantly as temperature is increased. Although the genotypes M1589, M1722 and 'Zero' have considerably different fatty acid compositions from that of Glenelg, their patterns of response to temperature are similar, indicating that the induced mutations they carry do not affect the thermal stability properties of the desaturation enzyme systems. The residual content of linolenic acid present in the 'Zero' genotype was not substantially altered by temperature treatments, indicating that the content in an

edible-quality linseed cultivar should be below 3% regardless of the temperature conditions experienced during seed maturation.

Effect of temperature on oleic and linoleic desaturation

The biosynthetic pathway leading to the production of linolenic acid in developing seeds is generally considered to involve the sequential desaturation of stearic acid to produce, progressively, oleic acid, linoleic acid, and eventually linolenic acid. The final two desaturation steps of fatty acid biosynthesis can be examined separately by considering the ratios ODR and LDR, which reflect the product/substrate relationship and are therefore directly proportional to the activity of the individual desaturase enzyme systems. In all four linseed genotypes, ODR was drastically reduced by increased temperature, being almost halved when temperature was raised from 15/10^{o}C to 21/16^{o}C (Figure 9). In contrast, LDR was relatively constant across the complete range of temperature (Figure 9). Thus the reductions in linoleic and linolenic acid contents observed at high temperatures are clearly due to the extreme temperature sensitivity of the oleic acid desaturation step, the desaturation of linoleic acid being temperature stable.

The contrasting temperature responses of these two desaturation reactions has not previously been reported in linseed or in any of the other major oilseed species containing linolenic acid such as soybean, rapeseed or mustard, probably because earlier studies were confined to consideration of the contents of individual fatty acids rather than their ratios. However, the effect of temperature on oleic acid desaturation

has been examined under controlled environment conditions in the premium quality edible oils sunflower and safflower, both of which contain substantial levels of oleic and linoleic, but no linolenic acid. Alteration to the proportions of oleic and linoleic acids in these species is therefore a direct indication of the temperature sensitivity of the oleic acid desaturation step. Canvin (1965) demonstrated that this desaturation step was temperature stable in the high-linoleic safflower cultivar Lethbridge 25, there being no significant reduction in linoleic acid content over the temperature range 10^{o}C to 27^{o}C. However, in the same study, the sunflower cultivar Sunrise proved to be highly temperature sensitive, linoleic acid declining linearly from 77% at 10^{o}C to 28% at 27^{o}C. Harris *et al.* (1978) observed a similar response for the sunflower cultivar Peredovik and concluded that the activity of the oleic acid desaturase enzyme system was reduced predominantly by increased minimum daily temperature. This conclusion was supported by Rochester and Silver (1983) on the basis of a greater incorporation of labelled CO_2 into linoleic acid when the night temperature was 15^{o}C compared with 22^{o}C. In view of the quite different thermal stabilities displayed by enzymes performing the same function in different species, the differential effect of temperature on the oleic acid and linoleic acid desaturases in linseed is not surprising.

Biochemical basis of temperature sensitivity

Harris and James (1969) concluded that the increase in desaturation activity at lower temperatures in sunflower and castor seeds was due to a greater availability of oxygen, which

is the rate-limiting factor for desaturation. This conclusion was based on studies of labelled acetate incorporation, in which it was observed that the reduction in desaturation activity at high temperatures could be overcome by raising the oxygen concentration. However, Harris and James found that this reversal did not occur with flax seeds, indicating that in this species oxygen availability is not the factor limiting desaturation activity at high temperatures. Presumably, the presence of photosynthetically-active chloroplasts in the seed tissue of flax removes the dependence on external gaseous oxygen. On the other hand, Bishop (1984) considers that the temperature-sensitive step controlling the level of linoleic acid in sunflower seed is not the desaturation of oleic acid but a later step involving the transfer of linoleic acid from phosphatidyl choline to triacylglycerol. Thus, the biochemical basis for the effect of temperature on fatty acid composition in flax seeds is currently unexplained.

Consequences for field performance

The sensitivity of fatty acid composition to temperature can be of considerable practical importance in commercial oilseed species. In some locations and some seasons the levels of linoleic acid in sunflower crops have been below the minimum industry standard for polyunsaturated margarine production (62%) due to the occurence of high temperatures during the seed maturation period (Harris *et al.*, 1978). The 'zero'-linolenic linseed genotype has a similar fatty acid composition to sunflower, but its response to temperature is not as dramatic, producing slightly less linoleic acid at temperatures below

24/19°C and more at temperatures above 24/19°C (Figure 10). In the current study the level of linoleic acid in 'Zero' fell below 62% when day/night temperature was greater than 18/13°C, approximately 3°C below the equivalent threshold for sunflower grown under similar controlled environment conditions (Harris *et al.*, 1978). When grown as a winter crop, linseed matures in the late September to early December period, during which the average daily maximum/minimum temperatures in typical southern Australian growing regions are generally below 22/12°C. Under these conditions the 'zero'-linolenic genotype should produce more than 62% linoleic acid and adequately meet industry standards. However, higher temperatures are regularly encountered in the northern linseed-growing districts such as the Darling Downs and northern NSW, and linoleic acid contents below 62% may occur if such genetic material was planted late in these regions.

Controlled environments differ from conditions experienced in the field in a number of respects. In the field diurnal temperature changes are gradual compared to the abrupt changes imposed in phytotron studies. Additionally both temperature and photoperiod increase as the season progresses in the field, but were kept constant throughout the maturation period in the present study. It might be expected that such differences would confound predictions of field performance based on controlled environment studies. However, Harris *et al.*, (1978) found a strong correlation between fatty acid composition of sunflower plants grown in the same phytotron as the current experiment with that of commercial crops maturing at similar temperatures. This was particularly noticeable for the threshold temperature

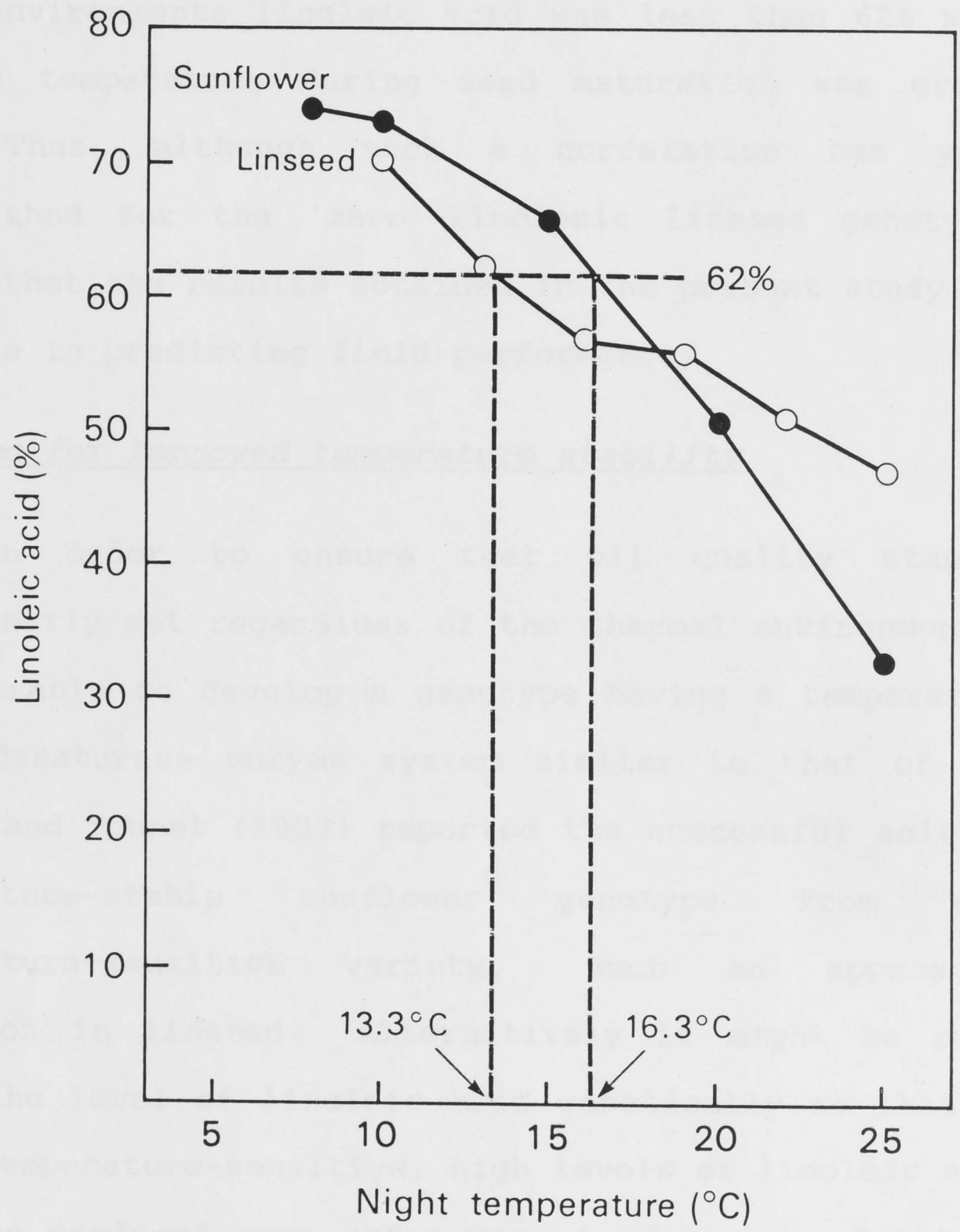


Figure 10: Relationship between night temperature and linoleic acid content in the linseed genotype 'Zero' (○—○) compared to that of the sunflower cv Peredovik (●—●) observed in the study of Harris *et al.* (1978).

required for linoleic acid content to meet industry standards. In the controlled environment study linoleic acid fell below 62% when night temperatures were greater than 16°C. Over a range of field environments linoleic acid was less than 62% where daily minimum temperature during seed maturation was greater than 17°C. Thus, although such a correlation has yet to be established for the 'zero'-linolenic linseed genotype, it is likely that the results obtained in the present study will prove reliable in predicting field performance.

Breeding for improved temperature stability

In order to ensure that oil quality standards are consistently met regardless of the thermal environment it would be desirable to develop a genotype having a temperature-stable oleic desaturase enzyme system similar to that of safflower. Downes and Tonnet (1982) reported the successful selection of a temperature-stable sunflower genotype from within a temperature-sensitive variety. Such an approach merits attention in linseed. Alternatively it might be possible to raise the level of linoleic acid genetically so that, although still temperature-sensitive, high levels of linoleic acid (>62%) could be produced even under warm conditions. In this respect it is encouraging to note that several linseed and flax genotypes have significantly higher ODR values than does Glenelg (see Chapter 2). If these high ODR values could be combined with the 'zero'-linolenic character, then the practical consequences of temperature sensitivity might be circumvented. A combination of these two approaches might result in a linseed genotype capable of very high levels of linoleic acid synthesis under all

temperature conditions and thus able consistently to meet the 72% requirement for the manufacture of highly-polyunsaturated (3:1) margarines.

CHAPTER FIVE

MATERNAL EFFECTS FOR SEED FATTY ACID COMPOSITION IN *L. USITATISSIMUM*

5.1. INTRODUCTION

The relative effects of maternal and embryo genotypes on the fatty acid composition of seed oil is important in inheritance studies and in determining the breeding procedure to be used in selection for specific fatty acid composition. It is also of general biochemical interest. The extent of these effects can be determined by the comparison of self-pollinated and reciprocally cross-pollinated seed borne on parental lines differing in fatty acid composition. Reciprocal F_1 seeds have identical nuclear genotypes, but differ in both the origin of their cytoplasm and the genotype of the parent on which they are borne. Such differences have no effect on oil composition in safflower (Knowles and Hill, 1964) or sesame (Mosjidis and Yermanos, 1984), indicating that genetic control is completely embryonic in these species. However, significant differences in the fatty acid composition of reciprocal F_1 seeds have been observed in other species such as corn (Jellum, 1966; Weber, 1983), soybean (Brim *et al.*, 1968), rapeseed (Thomas and Kondra, 1973), sunflower (Fernandez-Martinez and Knowles, 1982) and linseed (Yermanos and Knowles, 1962). These differences could have resulted either from their different cytoplasm or from an effect of the maternal parent.

The effect of cytoplasm can be examined by comparison of reciprocal F_2 seed populations, since although these are borne on genetically identical maternal parents (F_1) and have identical embryo genotype segregations, they have cytoplasms of different origin. Thomas and Kondra (1973) failed to detect any cytoplasmic effects in the F_2 generations of rapeseed crosses

and concluded that differences in the fatty acid composition of reciprocal F_1 seeds were due to true maternal effects. Where maternal effects have been reported in corn, soybean and flax, no attempts were made to determine whether such effects were truly maternal or cytoplasmic in nature.

The possible role that the endosperm fraction may play in influencing the composition of seed oil was not mentioned in any of the studies referred to above. Endosperm tissue is triploid and is formed by the combination of two maternal genomes and one paternal genome; thus, reciprocal F_1 seed, although having identical embryo genotypes (e.g. *Aa*) will have different endosperm genotypes (*AAa* and *Aaa*). Where fatty acid composition is controlled by additive genes, then dosage effects will cause the oil composition in the endosperm of the reciprocal F_1 seeds to differ and to be biased towards their maternal parents' phenotypes. If the endosperm contains an appreciable proportion of the total seed oil, then its differential fatty acid composition could be responsible for the observed differences in the composition of the total oil in reciprocal F_1 seed.

In linseed, the endosperm tissue contains approximately 20% of the total seed oil (Dybing, 1968; Dorrell, 1970) and additive gene action has been observed for fatty acid composition (see Chapter 4 and Yermanos and Knowles, 1962). Thus, the maternal regression for fatty acid composition of reciprocal F_1 seed of linseed observed by Yermanos and Knowles (1962) could have resulted from the differential composition of the endosperm fraction and not from a true maternal effect. In order to determine whether the maternal parent *per se* influences

the composition of seed oil it is necessary to remove the endosperm tissue and compare only the genetically identical embryos.

The aim of the present study was to determine whether there is a significant effect of maternal genotype on the fatty acid composition of linseed embryos. Since maternal effects, where reported, have generally differed in extent between crosses, three crosses were examined. The experiments were designed to detect reciprocal differences with greater precision than was possible in previous studies by using parents differing more widely in their fatty acid composition, and by attempting to minimise between-genotype environmental differences by having self-pollinated and cross-pollinated seed develop in the same capsule.

5.2. MATERIALS AND METHODS

The three crosses used were analysed sequentially. The results obtained influenced the methods used in making and analysing subsequent crosses. In each cross, when referring to hybrid genotypes, the first-mentioned line is the maternal parent.

Somaco x Avantgarde

This cross was performed and analysed in 1981, at which time the cvs Somaco and Avantgarde represented the extreme values for fatty acid composition in *L. usitatissimum* and thus afforded the best opportunity to examine maternal effects with

maximum precision. In order to eliminate the differences in micro-environments that might be experienced by selfed and crossed seed developing in separate capsules, mixed pollinations were performed by self-pollinating two stigmas and cross-pollinating the remaining three stigmas in each flower.

At maturity, individual capsules were harvested and embryos separated from the combined testa and endosperm by germinating seeds in the dark at room temperature (approx. 24^{o}C) for four days. Huber and Zalik (1963) reported that fatty acid composition of flax embryos did not change significantly during this period of germination; this result was confirmed during the course of this study. At four days, one cotyledon was removed from each seedling and analysed for fatty acid composition, and the remnant seedling was grown on to determine whether it was a hybrid or self, using the flower colour marker. The blue flower colour of cv. Somaco is conditioned by a single dominant gene. Thus, where cv. Avantgarde (white flowers) was the maternal parent, hybrid embryos gave rise to blue-flowered F_1 plants. Where cv. Somaco was the maternal parent, hybrid embryos were identified by the segregation of 3 blue : 1 white in the second generation (F_2). Only capsules containing at least two seeds of each class (hybrid and self) were included in the further analysis of data.

M1722 x Avantgarde

The discovery of the mutants M1589 and M1722 with greatly decreased levels of linolenic acid presented the opportunity to determine more precisely the effect of maternal genotype on embryo oil composition by examining a cross between parents that

differed much more than did Somaco and Avantgarde. In 1983, M1722 and cv. Avantgarde were crossed reciprocally. Because results from the Somaco x Avantgarde cross had indicated that between-seed variation was generally no less within capsules than between capsules, mixed pollinations were not performed, that is, capsules contained either all self or all hybrid embryos. All crosses were performed over a period of three days to ensure that capsules developed concurrently in identical environments, thus minimising between-capsule environmental variation. Self-pollinated flowers were also tagged during this three-day period. For each of the genotypes M1722, Avantgarde, M1722 x Avantgarde F_1, and Avantgarde x M1722 F_1, five seeds from each of four capsules were analysed individually. Seeds were germinated as for the previous cross, but as progeny testing was not required, analysis of fatty acid composition was performed on the whole embryo (endosperm and testa removed) rather than a single cotyledon.

Glenelg x 'Zero'

The isolation of the 'zero'-linolenic genotype referred to as 'Zero' in this study, by recombination of the M1589 and M1722 mutants of cv. Glenelg, allowed the effect of maternal genotype to be examined in the cross between cv. Glenelg and 'Zero', which appear to differ in only two genes determining fatty acid composition and have very similar, if not identical, cytoplasms. The reciprocal crosses were performed in 1984 on plants grown at each temperature in the Phytotron experiment described in Chapter 4, thus enabling the effect of temperature on maternal effect to be assessed. Over a period of four days

at each of the six temperature regimes, five reciprocal pollinations between cv. Glenelg and 'Zero' were performed and five naturally self-pollinated flowers of each parent tagged. From each of the 120 resulting capsules, five seeds were germinated as in the previous cross and their intact embryos bulked into a single sample per capsule for analysis of fatty acid composition. In addition, the combined testa and endosperm fractions from the 25 seeds of each genotype/temperature combination were bulked and analysed for fatty acid composition.

Fatty acid analyses

Fatty acid methyl esters were prepared and analysed by the the methods outlined in Chapter 1.3, but, because of small sample sizes, the reagent volumes were halved and 9 ml McCartney bottles used as reaction vessels. Where single embryos or cotyledons were analysed (Somaco x Avantgarde and M1722 x Avantgarde) manual injection of concentrated samples onto the GC column was necessary. However, the larger sample size in the bulked embryos of the Glenelg x 'Zero' cross permitted automatic injection.

Statistical analyses

Analysis of variance techniques were used to compare variation between seeds within capsules with that between capsules for each of the parental and reciprocal F_1 genotypes in the Somaco x Avantgarde and M1722 x Avantgarde crosses. In all three crosses, character means for each genotype were compared using the least significant difference procedure.

5.3. RESULTS

Somaco x Avantgarde

When data from all capsules was pooled, self embryos of cvs Somaco and Avantgarde differed significantly in content of all five fatty acids and in their ODR and LDR values (Table 20), thus allowing a comparison of self and hybrid embryos for all characters. These comparisons were performed both within capsules and on the data pooled over all capsules.

Six individual capsules for which cv. Avantgarde was the maternal parent were analysed. Of these, significant differences between self and F_1 embryos were evident for palmitic acid in all six capsules, for stearic acid or oleic acid in one, for linoleic acid or linolenic acid in two and for ODR and LDR in two and four capsules respectively (Table 20). When the data were pooled over capsules and a paired *t*-test performed, significant differences were evident for only palmitic acid, linoleic acid and LDR. Of the seven capsules for which cv. Somaco was the maternal parent, significant differences between self and F_1 embryos were evident for palmitic acid in three capsules, for linoleic acid in four, for stearic acid in one and for LDR in two. In no capsules did the self and F_1 embryos differ significantly in their contents of oleic acid or linolenic acid, or in their ODR value. As in the reciprocal cross, when pooled data were analysed, only palmitic acid, linoleic acid and LDR differed between the two embryo genotypes.

A comparison of the parental and reciprocal F_1 pooled

Table 20: Mean fatty acid composition for self and reciprocal F_1 embryos borne in the same capsules in the cross Somaco x Avantgarde.

Capsule number	Embryo type	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
				Avantgarde x Somaco				
1	Self(5)	5.1a	4.0	21.1a	12.8a	57.0a	3.43a	4.44a
	F_1(5)	6.0b	4.7	27.6b	14.1b	47.7b	2.33b	3.40b
2	Self(2)	5.3a	4.4	19.4	11.5a	59.7	3.80	5.22a
	F_1(5)	5.9b	4.7	17.5	13.3b	58.6	4.24	4.40
3	Self(4)	5.3a	4.0a	24.4	11.2	55.2	2.81	4.96
	F_1(2)	5.8b	4.8b	21.4	12.3	55.8	3.24	4.56
4	Self(4)	5.1a	5.5	21.3	11.7	56.4	3.21	4.84a
	F_1(2)	5.5b	5.4	21.2	12.3	55.8	3.23	4.55b
5	Self(2)	5.3a	4.2	17.4	12.7	60.6a	4.23a	4.77a
	F_1(5)	5.9b	4.1	24.0	13.3	52.8b	2.83b	3.99b
6	Self(2)	5.2a	3.8	28.2	12.7	50.3	2.24	3.98
	F_1(7)	5.6b	4.4	22.6	13.7	53.7	3.06	3.92
				Somaco x Avantgarde				
7	Self(7)	6.9	7.5	36.0	15.1	34.5	1.56	2.27
	F_1(2)	6.6	6.9	40.7	15.1	30.8	1.13	2.04
8	Self(7)	7.2a	7.5	32.5	16.5a	36.3	1.69	2.21
	F_1(3)	6.7b	6.7	32.7	14.2b	39.7	1.75	2.79
9	Self(6)	7.2a	5.6	34.6	15.3a	37.3	1.63	2.45a
	F_1(4)	6.7b	5.3	29.2	13.5b	45.5	2.23	3.37b
10	Self(6)	6.6a	6.0a	31.2	16.5a	39.9	1.94	2.42
	F_1(4)	5.7b	4.8b	42.1	12.5b	34.9	1.22	2.78
11	Self(5)	6.5	6.0	38.9	13.5	35.0	1.39	2.58
	F_1(4)	6.1	6.2	34.0	13.2	40.5	1.72	3.04
12	Self(6)	6.6	5.8	35.0	14.8	37.7	1.59	2.54a
	F_1(3)	6.2	5.6	33.6	13.9	40.8	1.75	2.93b
13	Self(2)	6.6	7.8	28.2	14.0a	43.7	2.16	3.12
	F_1(4)	6.1	7.0	36.5	11.8b	38.5	1.58	3.19
				Pooled data				
Avantgarde (19)		5.2a	4.4a	22.0a	12.1a	56.4a	3.25a	4.70a
Av. x Som. (26)		5.8b	4.6a	22.7a	13.4b	53.6a	3.13a	4.02b
MP value		6.0	5.5	28.1	13.7	46.8	2.46	3.57
Som. x Av. (24)		6.3c	6.0b	35.3b	13.3b	39.2b	1.66b	2.95c
Somaco (39)		6.8c	6.6b	34.2b	15.3c	37.1b	1.66b	2.43d

Within-capsules means followed by different letters are significantly different at the 5% level. Embryo numbers are bracketed.

means (Table 20) reveals that the levels of individual fatty acids in the embryo were influenced to different degrees by the genotype of the maternal parent. The contents of stearic acid, oleic acid and linolenic acid and the value of ODR in the reciprocal F_1 embryo populations were identical in each case to those of the respective maternal self embryo populations, indicating that in the determination of these characters the maternal parent completely dominates the embryo genotype. In contrast, the contents of linoleic acid in the reciprocal F_1 embryo populations were identical and corresponded to the mid-parent value, demonstrating that the embryo genotype determines this character without any maternal influence. For palmitic acid and LDR, the reciprocal F_1 embryo populations are significantly different from each other and from their respective maternal parents, the mean values for each F_1 population being intermediate between the mid-parent and maternal parent values. Thus both the maternal genotype and the embryo genotype exert an influence over the determination of these two characters in the embryo.

The relative magnitude of between- and within-capsule variation for all characters was examined individually for each of the four genotypes using analysis of variance techniques (Table 21). Capsule effects were significant in approximately half of the comparisons, indicating that, within each genotype, between-embryo differences within capsules were generally no less than those between capsules. Thus, comparisons between self and hybrid embryos borne in the same capsule did not consistently result in increased precision. This result explains why for some characters that differed significantly

Table 21: *F* ratios for comparison of between-capsule (numerator) and within-capsule (denominator) variation in fatty acid composition for parental and reciprocal F_1 genotypes in the cross Somaco x Avantgarde.

Genotype	Fatty acid 16:0	18:0	18:1	18:2	18:3	ODR	LDR
Avantgarde	0.64	6.54*	2.88	4.84*	2.97	2.82	5.49*
Avant. x Somaco	3.33*	2.32	3.87*	4.24*	4.57*	4.12*	5.82*
Somaco x Avant.	15.36*	6.13*	0.84	2.32	1.01	0.92	2.17
Somaco	5.87*	6.01*	0.46	4.05*	0.72	0.65	1.91

* Significant at the 5% level.

Table 22: Mean fatty acid composition for self and reciprocal F_1 embryos in the cross M1722 x Avantgarde.

Genotype	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
M1722	5.4^a	5.3^a	45.7^a	26.8^a	16.8^a	1.01^a	0.62^a
M1722 x Avant.	5.0^b	4.3^b	46.1^a	18.7^b	25.9^b	1.04^a	1.38^b
MP value	5.0	4.4	35.2	19.5	36.2	1.90	2.60
Avant. x M1722	4.8^c	2.9^c	26.1^b	20.6^c	45.6^c	2.61^b	2.22^c
Avantgarde	4.5^d	3.4^d	24.6^c	12.1^d	55.5^d	2.78^b	4.57^d

Means in the same column having superscript in common are not significantly different at the 5% level.

between self and F_1 embryos on the pooled data, there were individual capsules in which numerically large differences were not significant (e.g. for LDR in capsules 8, 10 and 11).

Hence, in order to compare self and F_1 genotypes with maximum precision, it is not necessary to have both genotypes developing within the same capsule. In view of the greatly increased time and effort needed to perform and progeny test mixed pollinations and the lack of advantage in experimental precision, this approach was not adopted in subsequent crosses.

M1722 x Avantgarde

Parental and reciprocal F_1 genotype means for the cross between M1722 and cv. Avantgarde are presented in Table 22. The parental self embryo populations differed significantly in their contents of all five fatty acids, and consequently in their values of ODR and LDR, although the difference was small for palmitic and stearic acid content. Reciprocal F_1 embryo populations also differed significantly in all characters indicating that the maternal parent exerted a significant effect over the determination of fatty acid composition.

The magnitude of the maternal effect varied between characters. Although the reciprocal F_1 embryo populations differed significantly in their contents of palmitic and linoleic acids, the difference was small relative to the parental differences, demonstrating that the influence of the maternal parent in the determination of these two fatty acids was slight. In contrast the maternal influence for both oleic acid content and ODR was complete, there being no significant

difference between the reciprocal F_1 embryo populations and their respective maternal self embryo populations. For linolenic acid content, the reciprocal F_1 embryo populations were intermediate between the mid-parent value and the respective maternal parents, indicating that embryo and maternal genotype influence the content of this fatty acid equally. Performance in respect of stearic acid and LDR differed in the reciprocal crosses. For LDR, the reciprocal F_1 populations were both intermediate between the mid-parent value and that of the M1722 self embryo population. For stearic acid, the M1722 x Avantgarde F_1 was equivalent to the mid-parent value, whereas the reciprocal F_1 was outside the parental range, being significantly less than the Avantgarde self embryo population.

A comparison of between-capsule and within-capsule variation in fatty acid composition of each genotype revealed that between-capsule variation was significantly greater than within-capsule in only 4 out of 28 comparisons (Table 23).

Table 23: *F* ratios for comparison of between-capsule (numerator) and within-capsule (denominator) variation in fatty acid composition for parental and reciprocal F_1 genotypes in the cross M1722 x Avantgarde.

Genotype	Fatty acid					ODR	LDR
	16:0	18:0	18:1	18:2	18:3		
M1722	0.04	3.68*	0.70	0.67	1.30	0.85	1.90
M1722 x Avant.	6.73*	1.09	1.10	1.57	0.70	1.22	0.41
Avant. x M1722	1.85	1.74	0.53	9.99*	0.33	0.35	0.85
Avantgarde	1.24	4.75*	0.29	0.58	0.50	0.29	1.15

* Significant at the 5% level.

Glenelg x 'Zero'

In both previous crosses (Somaco x Avantgarde and M1722 x Avantgarde) individual seeds of the same genotype differed little in fatty acid composition. Therefore analyses in the Glenelg x 'Zero' cross were performed on a bulk sample of five embryos per capsule, which provided sufficient material for automatic injection of samples onto the gas chromatograph column. This allowed more samples to be analysed and thus facilitated the examination of the cross at five different temperatures.

The mean fatty acid composition of the parental self and reciprocal F_1 embryo populations at each temperature are presented in Table 24. Self embryos of Glenelg and 'Zero' differed significantly in their content of palmitic, linoleic and linolenic acids and in their LDR value at all temperatures. Maternal effects were evident for these characters at some temperatures, the reciprocal F_1 embryos differing significantly in linoleic acid content at 15/10^{o}C and 24/19^{o}C, in linolenic acid content at 15/10^{o}C and 27/22^{o}C, and in LDR at 24/19^{o}C. However, in each case the difference was not large relative to that between the F_1 embryos and their respective maternal selfs. The small parental differences for palmitic acid content made it difficult to assess the extent of maternal influence over the determination of this character.

Significant differences between Glenelg and 'Zero' for stearic and oleic acid contents and ODR were observed only at lower temperatures, *viz.* 15/10^{o}C and 18/13^{o}C. For each of these

Table 24: Mean fatty acid composition of parental and reciprocal F_1 embryos in the cross Glenelg x 'Zero' grown at several temperatures.

Temperature and genotype	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
15/10°C							
Glenelg	4.9ab	5.6a	22.6a	21.9a	45.2a	2.98a	2.10a
Glenelg x Zero	5.3a	5.5a	22.1a	33.3b	33.8b	3.11a	1.03b
Zero x Glenelg	4.8b	3.4b	15.0b	39.2c	37.6c	5.14b	0.96b
Zero	5.3a	3.4b	17.6b	71.8d	1.9d	4.32b	0.03c
18/13°C							
Glenelg	5.0a	4.4a	36.7a	18.5a	35.4a	1.53a	1.95a
Glenelg x Zero	5.2b	3.6b	26.5b	35.6b	29.1b	2.47b	0.82b
Zero x Glenelg	5.1ab	4.0c	28.2b	35.8b	27.0b	2.25b	0.75b
Zero	5.2b	3.6b	30.4b	59.3c	1.5c	2.05ab	0.02c
21/16°C							
Glenelg	5.5a	5.1	40.0	16.8a	32.6a	1.26	1.96a
Glenelg x Zero	5.8ab	5.2	37.0	29.1b	22.8b	1.56	0.77b
Zero x Glenelg	5.7a	5.0	40.0	29.0b	20.3b	1.29	0.69b
Zero	6.1b	4.9	40.0	47.4c	1.5c	1.30	0.03c
24/19°C							
Glenelg	6.4a	8.0	28.2	15.1a	42.3a	2.04	2.81a
Glenelg x Zero	6.4a	8.5	25.0	28.3b	31.8b	2.47	1.12b
Zero x Glenelg	6.1b	7.6	25.7	31.9c	28.6b	2.43	0.90c
Zero	6.7c	7.8	24.6	59.0d	1.9c	2.51	0.03d
27/22°C							
Glenelg	6.7a	8.8	44.1	15.1a	25.3a	0.95	1.68a
Glenelg x Zero	7.0ab	9.5	34.1	26.3b	23.0a	1.45	0.88b
Zero x Glenelg	7.1ab	8.4	40.3	26.4b	17.7b	1.16	0.66b
Zero	7.3b	8.9	38.9	40.6c	4.3c	1.37	0.11c

Within temperatures, means in the same column having a letter in common are not significantly different at the 5% level. Means without annotation indicate non-significant genotype effects.

characters the reciprocal F_1 embryos were significantly different from each other but not from their respective maternal self embryos when grown at 15/10°C. At 18/13°C, reciprocal F_1 embryos were significantly different in stearic acid content, but not in oleic acid content or ODR.

The fatty acid composition of the combined endosperm and testa tissues was also examined in this cross. Although the testa is maternal tissue, it was included in the sample because its adherence to the solid endosperm made separation of the two tissues difficult, and the testa is known to contain very little oil (Dybing, 1968). Analysis of the combined tissue should therefore provide a good measure of the fatty acid composition of the endosperm lipids. The results presented in Table 25 show that the parental and reciprocal F_1 genotypes had similar contents of palmitic, stearic and oleic acids and similar ODR values. In contrast, the four genotypes differed considerably in their contents of linoleic and linolenic acids, and in their LDR values at each temperature. Because endosperm tissue is triploid, reciprocal F_1 endosperms are not genetically identical. Thus, with respect to the induced mutations, the Glenelg x 'Zero' F_1 endosperm has the genotype $Ln1^1Ln1^1Ln1^0\ Ln2^1Ln2^1Ln2^0$ and the 'Zero' x Glenelg F_1 endosperm is $Ln1^1Ln1^0Ln1^0\ Ln2^1Ln2^0Ln2^0$. The difference in the fatty acid composition of these two endosperms could therefore be due either to their different genotypes or to the influence of the maternal parent. This study could not separate these effects, but it seems more likely that the principal effect is that of the endosperm genotype since a comparison of the LDR values of the four endosperm genotypic classes clearly reveals the

Table 25: Fatty acid composition of parental and reciprocal F_1 endosperms in the cross Glenelg x 'Zero' grown at several temperatures.

Temperature and genotype	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
15/10°C							
Glenelg	10.5	1.6	13.1	17.3	57.3	5.69	3.31
Glenelg x Zero	10.4	1.5	13.2	23.8	50.8	5.65	2.13
Zero x Glenelg	10.0	1.4	13.8	37.2	37.5	5.41	1.01
Zero	10.6	1.6	14.9	69.5	3.4	4.89	0.05
18/13°C							
Glenelg	9.0	1.6	15.7	16.4	57.3	4.69	3.49
Glenelg x Zero	9.9	2.0	16.4	25.1	46.4	4.36	1.85
Zero x Glenelg	10.2	1.6	16.8	35.6	35.5	4.23	1.00
Zero	10.8	1.7	16.6	69.4	1.3	4.26	0.02
21/16°C							
Glenelg	10.8	1.8	20.3	20.6	46.0	3.28	2.23
Glenelg x Zero	10.1	1.9	19.9	23.3	44.8	3.42	1.92
Zero x Glenelg	10.3	1.8	18.5	37.1	32.3	3.75	0.87
Zero	11.1	1.5	17.9	68.8	0.7	3.88	0.01
24/19°C							
Glenelg	12.0	2.4	25.0	16.4	44.0	2.42	2.68
Glenelg x Zero	11.4	2.1	22.7	22.1	41.2	2.79	1.86
Zero x Glenelg	10.2	2.4	22.2	37.4	27.6	2.93	0.74
Zero	10.4	2.2	20.6	62.8	3.7	3.23	0.06
27/22°C							
Glenelg	10.3	2.7	26.6	14.1	45.9	2.26	3.26
Glenelg x Zero	10.1	3.1	26.6	20.9	39.0	2.25	1.87
Zero x Glenelg	10.7	2.6	24.5	33.8	28.3	2.53	0.84
Zero	10.4	2.7	24.2	58.6	3.7	2.57	0.06
Mean							
Glenelg	10.5	2.0	20.1	17.0a	50.1a	3.67	2.99a
Glenelg x Zero	10.4	2.1	19.8	23.0b	44.4a	3.69	1.93b
Zero x Glenelg	10.3	2.0	19.2	36.2c	32.2b	3.77	0.89c
Zero	10.7	1.9	18.8	65.8d	2.6c	3.77	0.04d

Means in the same column having a letter in common are not significantly different at the 5% level. Means without annotation indicate non-significant genotype effects.

additivity of the alleles at the *Ln1* and *Ln2* loci, genotypes with 0, 2, 4 and 6 normal alleles having LDR's of 0.04, 0.89, 1.93 and 2.99 respectively.

A comparison of the data in Tables 24 and 25 indicates that, for each of the four genotypes, endosperm and embryo tissue differ in their fatty acid composition, but lack of replication of endosperm data precluded statistical analysis. At all temperatures endosperm had lower contents of stearic and oleic acids and higher palmitic acid content and ODR. In Glenelg and the reciprocal F_1s, LDR was greater in the endosperm tissue, resulting in higher linolenic and lower linoleic acid content, but this relationship was less stringent at higher temperatures. In 'Zero', LDR and linolenic acid content differed little between the embryo and endosperm tissue. Linoleic acid, although slightly lower in endosperm tissue at 15/10^{o}C, was considerably and increasingly greater at higher temperatures, reflecting the lower temperature sensitivity of ODR in the endosperm compared to the embryo.

5.4. DISCUSSION

Maternal effects for fatty acid composition

The comparison of self and reciprocally cross-pollinated seed in three crosses between linseed genotypes differing widely in their fatty acid composition revealed that the maternal genotype on which seeds were borne influenced the fatty acid composition in the embryo. In all three crosses this influence was complete for oleic acid, the content in the embryo being determined solely by the maternal parent, but the magnitude of the maternal effect for the other fatty acids varied between crosses. Linoleic acid content was determined completely by the embryo genotype in the Somaco x Avantgarde cross and in the Glenelg x 'Zero' cross grown at 18/13^{o}C, but a partial maternal effect was apparent in the latter cross at 15/10^{o}C and in the M1722 x Avantgarde cross. Linolenic acid was controlled by the embryo genotype in the Glenelg x 'Zero' cross, by the maternal genotype in the Somaco x Avantgarde cross, with only a partial maternal effect in the M1722 x Avantgarde cross. Although there was only limited parental variation in content of the saturated fatty acids palmitic and stearic, partial maternal effects were observed in each cross.

Maternal effects for oleic and linoleic desaturation

The tendency for maternal effects on the polyunsaturated linoleic and linolenic acids to be smaller than on oleic acid is probably due to the contrasting effect of maternal parent on the oleic and linoleic desaturase systems. In each cross the desaturation of oleic acid to linoleic acid, as measured by ODR,

was under complete maternal control, whereas the further desaturation to linolenic acid, measured by LDR, was under embryo control and only slightly affected by the maternal parent. Previous reports of maternal effects in oilseed species have not considered the effects on the individual desaturation steps.

This study did not examine F_2 seed populations to determine to what extent the observed differences in fatty acid composition of reciprocal F_1 embryos were due to the different origins of their cytoplasms rather than to true maternal effects. However, since 'Zero' was derived directly from Glenelg their cytoplasms must be very similar if not identical. The presence of reciprocal F_1 differences for linoleic desaturation in crosses involving parental lines differing in the origin of their cytoplasm, when such differences were absent in the Glenelg x 'Zero' cross, might therefore indicate that cytoplasmic effects rather than true maternal effects were responsible. This would be in contrast to the conclusion of Thomas and Kondra (1973) that significant reciprocal F_1 differences observed for fatty acid composition in rapeseed were not due to cytoplasmic effects. Comparison of reciprocal F_2 populations from the Somaco x Avantgarde and M1722 x Avantgarde crosses are necessary to resolve this point.

Comparison of embryo and endosperm composition

The fatty acid composition of the endosperm tissue in the Glenelg and 'Zero' parents was consistently different from that of the embryo at all temperatures. In both genotypes, endosperm tended to have lower stearic and oleic acid contents, and higher

palmitic, linoleic and linolenic acid contents than did the embryo. Although the endosperm's higher palmitic and lower stearic acid content have been reported previously (Dorrell, 1970) the higher level of desaturation (both ODR and LDR were consistently greater in the endosperm) has not. It is unlikely to be a result of the higher ploidy level of the endosperm tissue because in the homozygous parental genotypes gene balance is maintained between the two tissues. On the other hand the triploid endosperms of the reciprocal F_1s are genetically different for the loci controlling linoleic and linolenic acid contents and their fatty acid compositions clearly indicate the existence of additive dosage effects at the *Ln1* and *Ln2* loci in endosperm tissue.

The different fatty acid compositions of reciprocal F_1 endosperms introduces a potential bias into the assessment of maternal effects in linseed based on whole seed analyses, as in the study of Yermanos and Knowles (1962). Consider for example the analysis of LDR in the Glenelg x 'Zero' cross at 15/10^{o}C. Here there was clearly no significant difference between the reciprocal F_1 embryos, the Glenelg x 'Zero' F_1 being 1.03 and the 'Zero' x Glenelg F_1 0.96. Based on the assumption that endosperm accounts for 20% of the total seed oil (Dybing, 1968), and that the LDR of the endosperm of the Glenelg x 'Zero' F_1 is 2.13 compared to 1.01 in the 'Zero' x Glenelg F_1, the LDR value of whole seed can be calculated as 1.25 in Glenelg x 'Zero' and 0.97 in 'Zero' x Glenelg. This substantial difference could lead to the erroneous conclusion that the maternal parent was influencing the determination of LDR in the seed. Similarly, in cases where maternal effects on embryo fatty acid composition do

occur, analysis of whole seed could lead to exaggerated estimates of their magnitude.

Selection for fatty acid composition

The presence of significant maternal effects has practical implications for selection for content of individual fatty acids in genetically-segregating populations. The development of the half-seed technique of determining the fatty acid composition on a single cotyledon and growing the remaining seed on enables selection to be performed on F_2 seed borne on an F_1 plant. This technique is now in routine use for early generation selection in rapeseed breeding programs (Downey and Harvey, 1963). The results of the current study indicate that in linseed such selection would be ineffective for oleic acid content and ODR, since both of these characters are controlled by the maternal parent, not the embryo. Since only partial maternal effects occur for linoleic acid, linolenic acid and LDR, selection for these characters should be effective, depending on the differences in parental phenotypes compared with the level of between-seed within-genotype environmental variation.

Of particular interest is selection for linolenic acid content in crosses between high-linolenic and 'zero'-linolenic genotypes aimed at backcrossing the edible oil quality characteristic into existing well-adapted linseed cultivars. Where LDR is controlled by only two genes in such crosses (as in the Glenelg x 'Zero' cross), the backcross embryos borne on the F_1 and subsequent backcross plants will be segregating 1 $Ln1^1Ln1^1Ln2^1Ln2^1$: 1 $Ln1^1Ln1^0Ln2^1Ln2^1$: 1 $Ln1^1Ln1^1Ln2^1Ln2^0$: 1 $Ln1^1Ln1^0Ln2^1Ln2^0$. These genotypes have LDR expectancies of 2.33,

1.42, 1.44 and 0.86 on the basis of results reported in Chapter 3. On the basis of the partial maternal effect observed in the Glenelg x 'Zero' cross under some temperature conditions, it would be predicted that each of the embryo genotypes would regress either towards the $Ln1^1Ln1^1.Ln2^1Ln2^1$ phenotype, if the F_1 plant were used as the male parent, or towards the $Ln^1Ln1^0.Ln2^1Ln2^0$ phenotype if it were used as the female parent. However, the ranking of the genotypes should remain the same and the magnitude of the expected differences in LDR value should still be sufficient to identify the double heterozygotes by selecting the embryos having lowest LDR value.

The validity of this prediction will depend on the amount of variation between embryos within genotypes. At most temperatures in the present study the coefficient of variation for LDR between genetically identical Glenelg x 'Zero' F_1 embryos was between 9% and 12%. Variation of this magnitude would not result in an overlap in LDR distributions for the genotypic classes in segregating backcross embryo populations. The testing of these predictions will require the analysis of backcross embryos by the half seed technique and the confirmation of their genotypes by progeny testing. Such experiments could not be completed during the course of this study.

CHAPTER SIX

GENERAL DISCUSSION

Biosynthesis of linolenic acid

Genetic control of linoleic and linolenic acid

Biosystematic implications

Conversion of linseed into an edible oil

Biosynthesis of linolenic acid

Based on biochemical studies it is generally accepted that linolenic acid in seed oils is synthesised by the succesive desaturations of oleic acid to linoleic acid and of linoleic acid to linolenic acid (Dutton and Mounts, 1966; Cherif *et al.*, 1975; Appelqvist, 1980; Stumpf, 1984). The results of the present study in which mutants having greatly reduced levels of linolenic acid also had greatly elevated levels of linoleic acid, with little alteration to other fatty acids, further supports the assertion that linoleic acid is the immediate biosynthetic precursor of linolenic acid in *L. usitatissimum* seeds. Furthermore, the strong inverse relationship between these two fatty acids in the seed oils of wild *Linum* species suggests that this pathway operates in the genus as a whole.

Two alternative pathways have previously been postulated to account for the synthesis of linolenic acid in plants. Kannangara and Stumpf (1972) concluded that linoleic and linolenic acids in spinach leaves were both produced directly from oleic acid by separate pathways, with linolenic being formed by the concerted removal of four hydrogens. Under this hypothesis, a large reduction in linolenic acid would be expected to result mainly in increased oleic acid content, with a partial flow-on into the linoleic acid pathway; such a pattern was not observed in the mutant genotypes of the current study. The other suggested pathway is that linolenic acid (C18:3) is produced by elongation of *cis*-3,6,9-dodecatrienoic acid (C12:3) (Kannangara *et al.*, 1973). However, shorter chain trienoic acids were not produced in response to reductions in linolenic acid in

the current study. Likewise, Oulaghan and Wills (1976) could not detect any of the expected precursor compounds during linseed development. Thus it appears that neither of these two suggested pathways account to any significant extent for the synthesis of linolenic acid in seed storage lipids in *L. usitatissimum*.

Although the sequence of steps in the desaturation chain is clearly 18:1 --> 18:2 --> 18:3, purification and identification of the desaturase enzymes involved has not yet been reported. This is due to the difficulty in obtaining *in vitro* activity of these membrane-bound enzymes (Stumpf, personal communication) and the lack of clearly-defined substrates (Stumpf, 1984). Thus the question of whether, in linseed, both desaturation steps are controlled by a single enzyme system, or by separate systems, as appears to be the case in rapeseed (Rakow, 1973), is unresolved.

The current study revealed three important differences in oleic and linoleic desaturation that support the concept of separate enzymes controlling these reactions. Firstly, the pattern of oleic, linoleic and linolenic acids in the wild *Linum* species suggested that oleic and linoleic desaturation could be independently manipulated; this was confirmed by the induction of mutants having large reductions in LDR with ODR unchanged. Secondly, although oleic desaturation was considerably reduced by high temperatures during seed maturation, linoleic desaturation was remarkably temperature stable. Thirdly, oleic desaturation in the developing embryo was strongly influenced by the genotype of the maternal parent on which the seeds were

borne, but no such effects were apparent for linoleic desaturation. Each of these observations is best explained by hypothesising separate enzymes controlling the two desaturation reactions.

The origin of the 2% linolenic acid remaining in the recombinant mutant line is of interest. Thies (1970) noted that accumulation of linolenic acid occurs only in the seed of oil-bearing plants which during their development possess green and photosynthetically-active chloroplasts, such as those of the families *Cruciferae* (rapeseed, mustard), *Leguminosae* (soybean) and *Linaceae* (linseed). Theis also observed a positive correlation between the chlorophyll content and the amount of linolenic acid present in mutant rapeseed genotypes. Because linolenic acid is known to be the principal fatty acid of the chloroplast thylakoid membranes, he considered that genotypes devoid of linolenic acid were unlikely to be obtained in these species.

This conclusion has been vindicated by the fact that although linolenic acid has been reduced in breeding lines of rapeseed (Rakow, 1973; Robbelen and Nitsch, 1975), soybean (Hammond *et al.*, 1972; Wilcox *et al.*, 1984), and now linseed, in no case has it been eliminated. The 2 - 3% level reported in the 'Zero' linseed genotype in the current study is the lowest yet produced in these species; it may represent the amount of linolenic acid associated with chloroplast structure or resulting from chloroplast activity, and thus may be the biological limit to selection in such species. In this regard it is interesting to note that linolenic acid content was 3%

lower in a chlorophyll-deficient rapeseed mutant compared to that in normal lines (Stringam and McGregor, 1980). It is possible that linolenic acid has been completely removed from the storage lipids of 'Zero' but that the chloroplast lipids maintain their normal composition. Analysis of fractionated lipids would be necessary to resolve this point.

Such a suggestion also raises the question as to whether the linolenic acid present in the chloroplast is synthesised *in situ*, perhaps under the genetic control of the chloroplast and by a different pathway to that operating during triglyceride synthesis in the oleosomes. Thus, although the two previously mentioned alternative pathways, originally suggested to account for biosynthesis of linolenic acid in leaf chloroplasts, do not appear to be responsible for the majority of linolenic acid synthesis in seed tissue, the possibility that they may operate in seed chloroplasts cannot yet be dismissed.

Genetic control of linoleic and linolenic acid

The proportion of linolenic acid in linseed oil is determined by the extent of desaturation of, firstly, oleic acid, and secondly, linoleic acid. The latter desaturation step has been demonstrated to be controllable by mutations at the two unlinked loci, *Ln1* and *Ln2*. In view of the variation patterns previously reported for sunflower and safflower, it is likely that the initial desaturation of oleic acid can similarly be manipulated in linseed by a small number of major genes.

The existence of two unlinked genes acting additively and equally to reduce linolenic acid content in linseed, is

reminiscent of the genetic system controlling erucic acid synthesis in *Brassica* species. In the diploid species *B. campestris* (genomic formula AA), a single gene controls the elongation of oleic acid (C18:1) to form eicosenoic (C20:1) and subsequently erucic (C22:1) acids (Dorrell and Downey, 1964), whereas in the two alloploid species *B. napus* (AACC) and *B. juncea* (AABB), erucic acid is controlled by alleles at two unlinked loci (Downey, 1964b; Harvey and Downey, 1964; Kirk and Hurlstone, 1983). It has therefore been postulated that each of the basic diploid *Brassica* genomes (A,B,C) carry a single gene, possibly homologous, controlling the elongation of oleic acid (Kirk and Hurlstone, 1983). Since *L. usitatissimum* is also considered to have a polyploid origin, it could be similarly suggested that the *Ln1* and *Ln2* genes, controlling linoleic desaturation, represent duplication of a single major gene present in the basic n = 9 *Linum* genome.

The presence of major genes blocking individual steps in the biosynthesis of a range of fatty acids might indicate that these changes result from the inactivation of the structural genes for the synthesis of the relevent desaturase or elongase enzymes. On the other hand, Stumpf (1975) considers that the inability of safflower to produce linolenic acid in its seed oil is due not to the absence of effective linoleic desaturase genes, but to a regulatory system that represses their activity, since safflower leaf tissue produces appreciable quantities of linolenic acid. However, the consistent pattern of codominant gene action observed in linseed, safflower and *Brassica spp* is more typical of structural genes than of regulatory genes, for which an all-or-nothing (dominance) pattern might be expected.

An alternative explanation of the safflower situation, consistent with the results of the current study, could be that fatty acid composition of the seed and leaf chloroplast lipids are under separate genetic control to that of the seed storage lipids.

Biosystematic implications

The relationship between linolenic acid content and subgeneric classification of *Linum* species, apart from being taxonomically useful, may also indicate evolutionary trends within the genus. Wild species of sect. *Linum* are considered to be the oldest species of the genus, having perennial growth habit, heterostylous flower structure and simple sculpturing of pollen exine, all of which are considered to be primitive rather than advanced characteristics (Saad, 1961; Rogers, 1963; Xavier and Rogers, 1963; Ockendon, 1968). The *L. perenne* complex of species (n=9) appears to be the basic type in this section with other species being derived from it by combinations of polyploidy and aneuploidy (Harris, 1968; Durrant, 1976). Since all of the species in sect. *Linum* contain high proportions of linolenic acid in their seed oils, the ability to desaturate linoleic acid may also be regarded as a primitive attribute in this genus.

The existence of clear groupings of high, medium and low linolenic types among species from other sections (see Table 3), indicates that during evolution the capacity to produce linolenic acid has been lost in a stepwise fashion. This pattern parallels the loss of function at either the *Ln1* or *Ln2* locus in M1589 and M1722, and at both loci in 'Zero', following

induced mutation in *L. usitatissimum* (Figure 11) and suggests that these genes, or other genes having similar effects, have been similarly inactivated on occasions during evolution. Such inactivation could have resulted from natural mutation, loss of chromatin or chromosomes carrying these genes, or combinations of these processes. This hypothesis cannot currently be tested further but could potentially be addressed if it were possible to obtain fertile interspecific hybrids involving high and low linolenic species, such as *L. usitatissimum* and *L. mucronatum*. Alternatively, if the mutants in this study prove to be null alleles of linoleic desaturase structural genes, then it may be possible to develop molecular probes that could be used to examine wild *Linum* species for the presence or absence of sequences homologous to the *L. usitatissimum* genes.

Conversion of linseed into an edible oil.

Across a wide range of post-flowering temperatures, linolenic acid content in the homozygous genotype 'Zero' was consistently lower than the 3% regarded as the upper limit desirable for an edible oil. At this level it is below the content found in both soybean and rapeseed. Additionally, results of the Phytotron experiment identified environmental conditions under which this genotype produced linoleic acid content above the 62% necessary for the manufacture of polyunsaturated margarines. These conditions, temperature during maturation below 21/16^{o}C, should be readily attained when linseed is grown as a winter-spring crop in temperate climates.

The 'Zero' genotype would appear to be agronomically suitable for use as a cultivar in those Australian environments

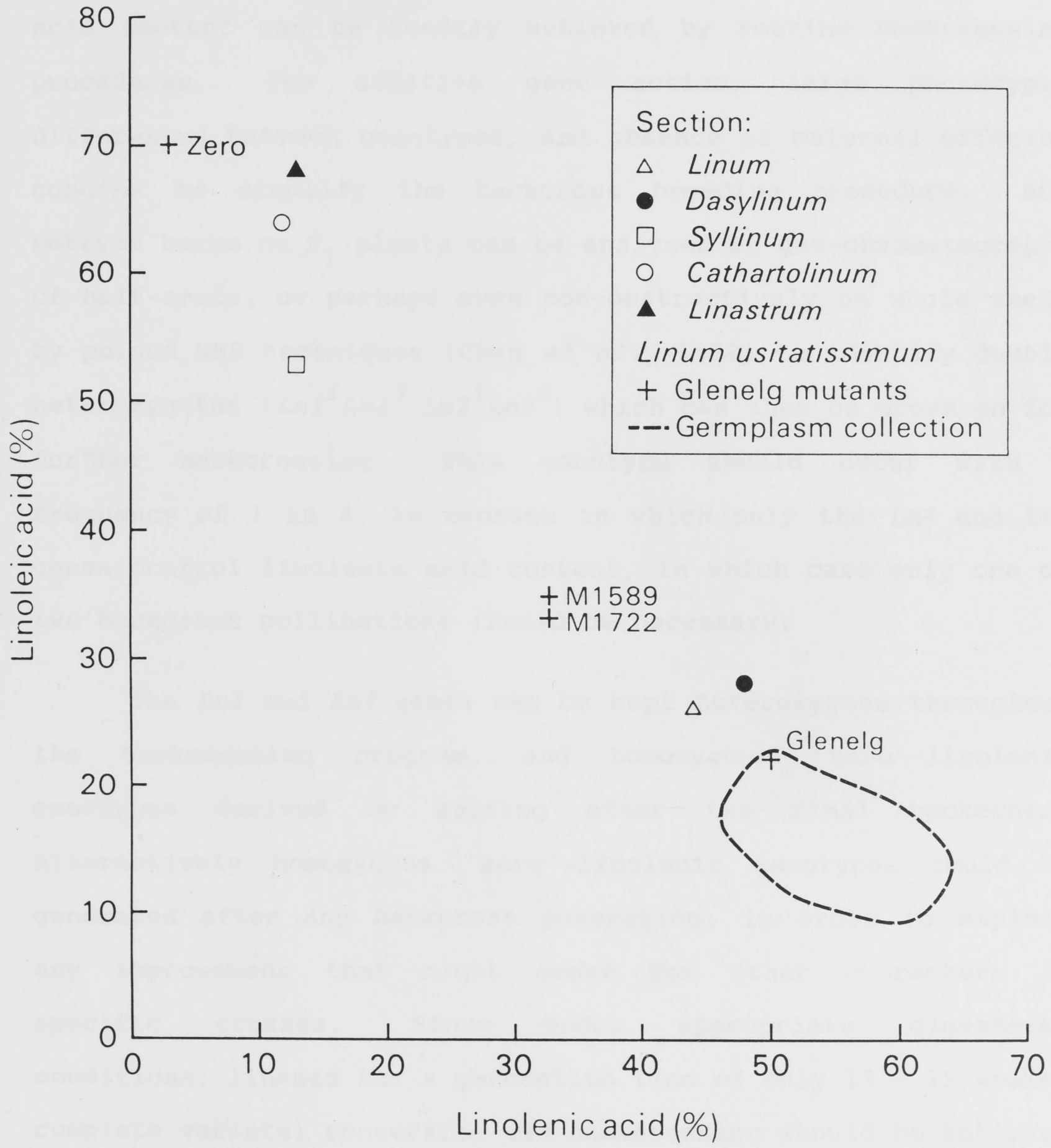


Figure 11: Relationship between linoleic acid and linolenic acid content in cv Glenelg and the mutant genotypes M1589, M1722 and 'Zero' compared to that in the taxonomic sections of the genus *Linum*.

in which Glenelg has been successfully cultivated. Conversion of cultivars adapted to other environments to 'zero'-linolenic acid content can be readily achieved by routine backcrossing procedures. The additive gene action, large phenotypic differences between genotypes, and absence of maternal effects, combine to simplify the backcross breeding procedure. BC_1 embryos borne on F_1 plants can be analysed by gas-chromatography of half-seeds, or perhaps even non-destructively on whole seeds by pulsed NMR techniques (Chen *et al.*, 1978) to identify double heterozygotes ($Ln1^1Ln1^0$ $Ln2^1Ln2^0$) which can then be grown on for further backcrossing. This genotype should occur with a frequency of 1 in 4, in crosses in which only the *Ln1* and *Ln2* genes control linolenic acid content, in which case only one or two backcross pollinations should be necessary.

The *Ln1* and *Ln2* genes can be kept heterozygous throughout the backcrossing program, and homozygous 'zero'-linolenic genotypes derived by selfing after the final backcross. Alternatively homozygous 'zero'-linolenic genotypes could be generated after any backcross generation, in order to exploit any improvement that might occur for other characters in specific crosses. Since under appropriate glasshouse conditions, linseed has a generation time of only 13 - 15 weeks, complete varietal conversion *via* backcrossing should be achieved within two years (excluding seed increase and evaluation). Furthermore, the absence of any large temperature effects on LDR obviates the need for strict temperature control during backcrossing.

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APPENDIX

Seed weight, oil content and fatty acid composition of the flax and linseed germplasm analysed in further detail in Chapter 2.

Entry no.	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
			Flax lines						
Flx01	423	34.0	5.4	2.2	14.6	16.5	61.5	5.34	3.73
Flx02	436	37.4	4.6	3.0	16.1	16.1	60.2	4.74	3.74
Flx05	430	37.9	5.4	3.8	15.4	14.3	61.1	4.90	4.27
Flx06	490	39.7	5.1	3.6	16.5	16.3	58.5	4.53	3.59
Flx07	420	42.2	4.8	2.6	14.3	14.1	64.2	5.48	4.55
Flx08	496	33.3	5.3	1.5	17.6	17.6	58.0	4.30	3.30
Flx09	523	41.2	5.6	3.3	16.5	13.8	60.8	4.52	4.41
Flx10	500	37.5	5.0	3.2	16.9	13.6	61.2	4.43	4.50
Flx12	493	37.4	5.4	3.1	17.1	15.2	59.3	4.36	3.90
Flx13	383	34.2	4.6	2.1	20.0	17.0	56.2	3.66	3.31
Flx14	476	37.6	5.1	2.1	19.4	17.3	56.1	3.78	3.24
Flx15	536	38.2	5.2	2.7	19.3	15.3	57.5	3.77	3.76
Flx16	360	35.1	5.0	1.9	19.6	17.9	55.7	3.76	3.11
			Linseed lines						
W1C	413	41.7	6.0	3.2	15.7	16.3	58.8	4.78	3.61
W2B	463	39.6	5.2	1.3	16.8	16.6	60.1	4.57	3.62
W4D	709	43.1	5.4	2.3	20.5	17.7	54.1	3.50	3.06
W4E	706	42.5	5.3	3.8	18.7	16.1	56.2	3.87	3.49
W5A	743	42.4	6.1	2.8	19.2	17.5	54.5	3.75	3.11
W7B	576	42.9	5.4	2.4	20.7	18.8	52.7	3.45	2.80
W8B	673	41.7	6.2	2.7	17.9	15.6	57.7	4.09	3.70
W9B	669	43.0	6.7	3.4	17.6	13.8	58.5	4.11	4.24
W10C	653	40.8	7.7	3.6	16.4	14.9	57.5	4.41	3.86
W11C	616	41.2	6.7	1.3	21.4	19.5	51.1	3.30	2.62
W11D	579	43.0	5.7	1.6	19.7	18.5	54.6	3.71	2.95
W12A	566	44.3	4.3	1.6	17.8	18.0	58.5	4.30	3.25
W12B	599	39.5	6.1	3.4	20.1	17.5	53.0	3.51	3.03
W14A	506	40.7	5.1	1.7	16.6	17.3	59.4	4.62	3.43
W15A	526	42.8	6.6	2.6	15.5	15.6	59.7	4.86	3.83
W15B	523	38.1	5.4	1.8	17.7	19.5	55.7	4.25	2.86
W24A	639	43.1	6.1	2.1	18.9	16.9	56.0	3.86	3.31
W25A	589	39.7	6.6	2.5	13.8	15.9	61.3	5.59	3.86
W25B	599	44.5	8.0	4.3	19.8	16.3	51.6	3.43	3.17
W25C	556	41.1	8.0	4.1	20.2	15.6	52.2	3.36	3.35
W26A	679	44.3	5.9	2.4	19.4	17.7	54.6	3.73	3.08
W35B	643	46.0	8.1	4.2	19.0	18.2	50.6	3.62	2.78
W38A	569	40.3	5.2	2.1	19.7	17.8	55.3	3.71	3.11

Entry no.	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
W45A	609	41.4	6.4	2.8	18.0	16.9	56.0	4.05	3.31
W53A	466	41.3	5.4	3.4	16.9	15.2	59.1	4.40	3.89
W54A	516	42.7	7.2	2.9	19.3	19.3	51.8	3.68	2.68
W64A	792	41.6	6.6	4.2	18.5	15.8	55.0	3.83	3.48
W65A	846	43.8	9.0	3.0	20.9	18.9	48.3	3.22	2.56
W69A	889	43.1	7.9	3.3	15.8	15.9	57.1	4.62	3.59
W71A	726	43.1	7.4	3.7	16.8	15.6	56.5	4.29	3.62
W71B	703	45.5	8.2	3.7	17.9	15.0	55.3	3.93	3.69
W73A	629	45.0	8.1	3.3	17.3	15.6	55.7	4.12	3.57
W74A	643	45.0	7.7	3.4	23.5	15.5	49.9	2.78	3.22
W78H	403	38.8	6.1	2.0	18.9	18.7	54.4	3.87	2.91
W79A	486	40.5	6.4	1.9	14.0	17.7	59.0	5.48	3.33
W80A	666	40.8	7.1	3.2	15.8	15.5	58.5	4.68	3.77
W81A	563	43.0	8.1	3.6	18.6	14.7	55.1	3.75	3.75
W82D	659	40.9	7.8	2.7	18.7	15.0	55.8	3.79	3.72
W83A	490	42.0	6.0	2.1	15.0	16.0	60.8	5.12	3.80
W84A	536	43.2	7.0	3.5	17.9	14.9	56.9	4.01	3.82
W85A	719	44.4	7.2	3.1	17.1	14.7	57.9	4.25	3.94
W86A	766	41.8	7.9	3.2	17.1	17.2	54.6	4.20	3.17
W87B	653	43.2	6.3	2.2	18.2	18.5	54.8	4.03	2.96
W88A	666	42.0	8.3	3.0	17.8	15.8	55.3	3.99	3.50
W88B	589	40.8	8.1	3.3	18.7	19.8	50.1	3.74	2.53
W89A	453	38.3	6.4	1.7	17.2	17.7	57.1	4.35	3.23
W93B	543	39.1	7.5	2.9	15.3	15.1	59.3	4.86	3.93
W101A	859	43.2	7.8	3.3	15.8	16.2	56.9	4.63	3.51
W105B	539	44.2	6.5	1.9	15.4	16.1	60.2	4.95	3.74
W105C	606	43.3	6.8	2.2	17.3	15.1	58.9	4.28	3.90
W109B	460	40.7	7.3	2.4	14.7	14.9	60.7	5.14	4.07
W122A	649	42.4	8.4	3.6	17.3	14.2	56.6	4.09	3.99
W124B	546	42.2	7.4	2.4	15.7	16.3	58.3	4.75	3.58
W125C	852	43.4	8.2	3.4	19.5	16.2	52.8	3.54	3.26
W127A	902	44.4	8.4	3.2	17.7	16.0	54.8	4.00	3.43
W130A	623	41.5	6.5	1.7	22.4	17.3	52.1	3.10	3.01
W130C	586	42.0	7.1	3.3	16.8	14.2	58.7	4.34	4.13
W130I	646	41.8	6.8	2.9	17.5	14.5	58.4	4.17	4.03
W131A	989	43.3	7.9	5.3	18.3	15.3	53.3	3.75	3.48
W133A	503	37.3	7.4	2.6	15.0	15.1	59.9	5.00	3.97
W134A	529	42.8	7.4	3.1	16.9	17.0	55.6	4.30	3.27
W135A	979	43.2	7.8	3.0	17.9	17.0	54.3	3.98	3.19
W135B	932	43.5	7.5	2.1	19.1	15.8	55.5	3.73	3.51
W140B	473	38.2	6.5	2.5	16.8	17.2	57.1	4.42	3.32
W140D	739	42.3	8.0	2.7	15.9	16.8	56.7	4.62	3.38
W140G	729	43.1	6.8	3.4	17.9	14.9	57.1	4.02	3.83
W143A	500	38.1	6.3	3.2	13.3	15.2	62.0	5.80	4.08
W145A	496	40.5	5.5	2.3	14.9	14.2	63.1	5.19	4.44
W148A	783	43.9	6.7	3.2	18.0	15.6	56.5	4.01	3.62
W152A	713	39.9	6.2	3.7	16.8	16.2	56.2	4.31	3.47
W153A	729	43.9	5.8	3.4	15.6	14.0	61.2	4.82	4.37
W154B	589	42.8	7.0	3.8	16.7	11.9	58.8	4.23	4.94
W154F	679	46.2	6.3	3.1	19.1	10.4	61.1	3.74	5.88
W155B	693	44.9	6.1	2.8	22.4	13.3	55.4	3.07	4.17
W157A	649	42.9	7.8	2.8	16.5	15.1	57.9	4.42	3.83
W157B	656	41.0	7.3	3.5	18.9	14.3	56.0	3.72	3.92

Entry no.	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
W158A	716	41.9	7.7	3.5	18.7	17.1	52.0	3.70	3.04
W158B	1009	43.3	7.5	3.0	18.4	16.0	55.3	3.88	3.46
W159A	523	38.2	7.3	2.1	14.7	13.7	62.3	5.17	4.55
W160A	536	38.9	7.9	2.4	17.1	12.8	59.9	4.25	4.68
W162A	543	40.3	6.5	2.5	14.6	17.0	59.4	5.23	3.49
W165A	477	37.3	6.9	3.4	17.9	14.4	57.4	4.01	3.99
W166B	703	43.3	7.2	3.0	18.8	18.4	52.7	3.78	2.86
W166D	699	43.5	7.7	4.6	19.4	17.3	51.0	3.52	2.95
W166E	763	42.9	6.2	2.2	18.8	18.4	54.4	3.87	2.96
W169A	506	39.5	5.7	1.4	17.5	17.4	58.1	4.31	3.34
W170A	619	43.3	6.5	3.4	18.2	13.4	58.5	3.95	4.37
W174A	613	43.0	6.5	2.0	19.3	17.9	54.4	3.75	3.04
W175A	533	38.4	6.9	2.6	16.7	17.7	56.2	4.43	3.18
W177A	809	44.0	6.2	1.6	22.1	18.3	51.9	3.18	2.84
W184A	706	42.4	7.1	3.4	17.0	14.3	58.3	4.27	4.08
W185A	456	34.6	4.7	1.3	17.2	17.8	59.1	4.47	3.32
W187A	553	44.5	6.4	2.5	17.9	17.4	55.9	4.09	3.21
W193C	816	42.6	6.1	1.9	22.6	18.8	50.7	3.08	2.70
W199A	589	40.2	7.2	3.3	18.2	15.5	55.8	3.92	3.60
W200A	563	36.8	6.5	2.4	16.1	16.6	58.4	4.66	3.52
W201A	490	36.0	5.5	3.0	16.6	15.6	59.4	4.52	3.81
W202A	866	43.5	6.3	3.5	20.7	15.7	53.9	3.36	3.43
W204A	549	43.6	6.1	3.2	16.2	17.2	57.4	4.60	3.34
W206A	559	42.0	6.3	2.9	16.3	16.8	57.6	4.56	3.43
W208A	656	43.5	7.3	3.0	18.9	17.1	55.9	3.86	3.27
W211A	493	39.9	6.0	2.7	17.4	14.0	59.9	4.25	4.28
W214A	626	42.2	6.0	3.1	17.6	15.7	57.6	4.16	3.67
W215A	649	44.2	8.4	4.6	18.8	18.9	49.5	3.64	2.62
W216A	599	41.8	7.0	3.2	19.7	19.0	51.1	3.56	2.69
W217C	663	43.9	5.6	3.4	15.8	15.7	59.6	4.77	3.80
W218A	723	40.8	8.4	4.0	20.3	16.3	50.9	3.31	3.12
W219A	773	39.7	6.5	2.9	17.1	16.5	57.0	4.30	3.45
W221A	553	37.5	7.3	3.0	17.9	18.8	53.0	4.01	2.82
W223B	746	41.8	7.0	3.7	15.6	14.4	59.3	4.72	4.12
W234A	649	39.8	6.2	3.5	17.0	16.4	57.1	4.32	3.48
W237A	892	41.7	6.6	2.4	21.9	15.7	53.4	3.16	3.40
W242A	716	41.1	5.7	2.0	18.9	17.4	56.1	3.89	3.22
W247B	629	42.9	5.6	1.5	18.4	18.8	55.7	4.05	2.96
W253B	483	35.4	6.6	4.0	17.1	20.9	51.5	4.23	2.46
W255A	526	35.1	5.7	4.7	17.2	18.2	54.3	4.22	2.98
W258A	606	42.0	7.1	2.5	18.3	15.7	56.5	3.95	3.60
W258C	519	42.5	7.6	3.1	16.7	17.7	55.0	4.35	3.11
W259A	546	44.8	7.7	3.3	17.0	17.2	54.9	4.24	3.19
W259B	569	42.8	7.6	3.2	16.8	16.9	55.5	4.31	3.28
W260A	616	42.7	7.0	2.9	19.1	17.3	53.8	3.72	3.11
W265A	766	45.4	8.6	4.4	20.5	16.6	49.9	3.24	3.01
W266A	546	40.3	7.8	3.6	17.0	17.1	54.5	4.21	3.19
W274B	593	40.3	5.9	1.4	20.3	18.4	54.0	3.57	2.93
W278A	739	42.6	7.2	5.4	21.0	16.9	49.1	3.14	2.91
W284A	932	41.7	7.6	4.4	21.1	15.4	51.5	3.17	3.34
W288A	538	40.7	7.9	4.7	23.4	12.1	51.8	2.73	4.28
W291B	749	40.5	8.0	4.4	18.8	14.6	54.3	3.66	3.72

Entry no.	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
W291C	496	35.5	5.5	1.6	19.6	17.0	56.2	3.73	3.31
W291E	916	44.6	9.0	4.9	17.9	15.8	52.4	3.81	3.32
W291F	763	42.7	8.6	5.4	19.0	15.3	51.7	3.53	3.38
W291G	886	43.4	7.8	4.2	22.1	16.2	49.7	2.98	3.07
W291I	716	44.6	8.4	5.0	21.5	15.9	49.2	3.03	3.09
W291J	796	43.5	9.1	4.6	16.8	15.9	53.7	4.14	3.38
W291Y	942	45.9	9.2	3.4	18.9	11.0	59.6	3.74	5.42
W292A	569	41.5	8.3	6.2	21.0	14.7	49.8	3.07	3.39
W292B	763	43.5	6.4	2.2	25.2	13.8	52.5	2.63	3.80
W294A	569	44.1	6.3	5.0	19.3	17.2	52.2	3.60	3.03
W295A	643	40.0	8.0	4.4	22.0	18.3	47.9	3.01	2.62
W296A	533	41.4	7.9	2.7	17.2	18.8	53.6	4.21	2.85
W307A	856	42.4	8.0	4.5	21.3	17.4	48.8	3.11	2.80
W308A	656	43.3	7.2	4.3	19.1	18.3	51.1	3.63	2.79
W309A	649	40.3	7.3	3.9	17.3	16.0	55.5	4.13	3.47
W310A	573	41.3	6.5	4.1	19.7	16.9	52.8	3.54	3.12
W311A	516	38.4	7.3	4.1	18.7	18.6	51.4	3.74	2.76
W312A	616	44.1	6.2	3.5	19.8	18.0	52.5	3.56	2.92
W313A	519	41.5	8.2	2.8	18.7	17.9	52.5	3.76	2.93
W314A	556	41.6	8.0	4.9	20.9	17.3	48.9	3.17	2.83
W315A	393	42.9	7.2	3.6	21.0	15.0	53.2	3.25	3.55
W316A	523	40.1	8.1	4.5	17.0	17.2	53.2	4.14	3.09
W317A	543	41.5	7.1	3.5	17.2	17.4	54.9	4.20	3.16
W318A	536	40.8	7.4	3.5	22.4	17.8	48.9	2.98	2.75
W319A	766	42.1	8.3	4.8	23.0	18.3	45.5	2.77	2.49
W320A	759	42.8	5.8	3.1	21.1	16.4	53.6	3.32	3.27
W322A	649	41.3	8.1	4.2	18.8	16.3	52.7	3.67	3.23
W323A	496	38.7	7.7	2.8	18.4	18.5	52.7	3.87	2.85
W324A	509	44.2	7.1	2.8	17.4	15.0	57.8	4.18	3.85
W326A	669	41.6	5.0	3.7	19.6	15.7	56.0	3.66	3.57
W328A	626	40.2	7.8	4.0	19.3	16.1	52.9	3.58	3.29
W329A	653	41.0	7.2	4.4	22.1	16.1	50.2	3.00	3.12
W332A	529	41.6	6.4	2.9	23.0	15.2	52.5	2.94	3.45
W333A	666	41.5	7.9	4.5	21.4	15.2	51.1	3.10	3.36
W334A	589	44.5	7.2	4.6	17.9	18.1	52.2	3.93	2.88
W336A	430	41.4	6.5	3.5	20.4	13.1	56.6	3.42	4.32
W338A	623	42.7	8.0	3.8	18.9	16.5	52.8	3.67	3.20
W339A	703	41.6	6.8	4.1	18.0	15.2	56.0	3.96	3.68
W340A	676	42.0	8.0	4.3	19.5	18.5	49.9	3.51	2.70
W341A	626	42.9	6.4	4.0	18.9	16.0	54.7	3.74	3.42
W343A	529	45.0	5.9	3.2	18.3	14.2	58.4	3.97	4.11
W344A	586	42.8	4.9	3.2	17.5	14.2	60.2	4.25	4.24
W345A	546	42.4	3.8	2.3	18.3	17.1	58.5	4.13	3.42
W346A	513	41.0	6.8	4.0	19.3	15.4	54.6	3.63	3.55
W348A	906	43.5	7.8	5.1	23.2	16.7	47.3	2.76	2.83
W349A	653	41.7	7.4	5.0	22.1	14.6	51.0	2.97	3.49
W350A	616	42.9	6.8	3.8	18.7	16.4	54.4	3.79	3.32
W351A	433	37.7	5.5	2.8	19.2	17.6	54.9	3.78	3.12
W352A	703	40.0	5.6	2.5	19.3	17.1	55.4	3.76	3.24
W353A	616	43.6	6.5	5.1	19.1	18.3	51.1	3.63	2.79
W354A	753	43.7	7.8	4.9	19.8	16.2	51.4	3.41	3.17
W355A	763	43.4	6.5	3.7	18.7	17.2	53.9	3.80	3.13

Entry no.	Seedwt (mg)	Oil (%)	Fatty acid composition (%) 16:0	18:0	18:1	18:2	18:3	ODR	LDR
W356A	633	43.3	8.9	3.1	17.7	15.0	56.7	4.05	3.78
W357A	519	42.4	7.5	3.1	17.7	15.0	56.7	4.05	3.78
W358A	543	41.7	7.4	3.8	18.4	18.9	51.5	3.83	2.72
W359A	596	42.0	7.4	4.8	17.4	14.0	56.4	4.05	4.03
W361A	486	41.5	7.5	4.0	19.5	16.0	52.9	3.53	3.31
W362A	546	43.0	7.4	3.0	18.1	13.8	57.7	3.95	4.18
W363A	753	43.8	7.2	5.3	19.6	15.4	52.5	3.46	3.41
W364A	573	42.6	6.7	3.9	18.2	14.3	56.9	3.91	3.98
W365A	616	42.8	7.5	5.8	21.7	15.7	49.4	3.00	3.15
W366A	699	45.5	7.2	3.8	19.8	17.2	52.0	3.49	3.02
W368A	736	43.1	5.6	2.6	21.1	17.4	53.3	3.35	3.06
W371A	659	46.4	7.7	4.5	23.8	11.8	52.1	2.68	4.42
W385A	500	38.0	8.0	3.8	16.9	16.7	54.7	4.22	3.28
W386A	539	39.7	6.2	2.5	20.1	16.0	55.2	3.54	3.45
W387A	906	43.1	7.7	5.1	21.9	17.4	48.1	2.99	2.76
W388A	559	43.1	6.1	3.6	20.0	15.6	54.7	3.52	3.51
W389A	506	43.3	6.7	3.2	20.5	15.0	54.6	3.40	3.64
W390A	709	40.5	7.3	4.0	20.5	13.6	54.6	3.33	4.01
W391A	606	43.7	6.0	3.5	17.3	14.7	58.5	4.23	3.98
W392A	676	44.2	7.4	4.4	21.3	15.1	51.8	3.14	3.43
W393A	646	44.5	6.4	3.8	16.2	13.5	60.1	4.54	4.45